Universidade de Lisboa

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Papel do peptídeo natriurético cerebral de tipo B na classificação do tipo de AVC isquémico

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Abstract

Even after an extensive etiological investigation in about one third of ischemic strokes it is not possible to establish a cause. A fraction of these strokes could have been due to an episode of paroxysmal atrial fibrillation which could not be identified. Studies show that the current available methods to diagnose paroxysmal atrial fibrillation have a low sensitivity. N-terminal of the brain natriuretic peptide (NT-proBNP) could be useful as a biomarker of cardioembolic stroke.

NT-proBNP serum levels were initially evaluated in 66 ischemic atroke patients. Patients with cardioembolic stroke had higher levels of NT-proBNP than patients with non-cardioembolic stroke. NT-proBNP had a very good accuracy to diagnose cardioembolic stroke related to atrial fibrillation (AUC-0.92) Two cut-off points of serum NT-proBNP levels with a high sensitivity (265.5 pg/mL) and specificity (912 pg/mL) for the diagnosis of paroxysmal atrial fibrillation were established. Although NT-proBNP levels started to decrease 72 hours after stroke onset, the diagnostic accuracy of NT-proBNP to diagnose cardioembolic stroke was similar in the first 72 hours after ischemic stroke. In a validation study performed in 184 patients the cut-off points of 265.5 pg / mL and 912.0 pg / mL of NT-proBNP had a sensitivity, specificity, positive predictive value and negative predictive value in the first study versus validation study of respectively (94.4% vs 100%, 72.9% vs 70.5%, 56.6% vs 59.1%, 97.2% vs 100%) versus (55.5% vs 81.8%, 97.9% vs 87.5, 90.9% vs 73.8%, 83.9 vs 91.9%) for the diagnosis of ischemic stroke of cardioembolic etiology associated to atrial fibrillation. A prospective study including 80 patients was done to evaluate the cut-off points in patients with cryptogenic stroke. The Area Under the Curve of the Receiver Operating Curve of NT-proBNP for the diagnosis of paroxysmal atrial fibrillation in patients with cryptogenic stroke was good - 0.83, 95% CI (0.73-0.92), the cut-off point of 265.5 pg/mL had a sensitivity of 88.2% 95% CI (65.7-96.7%). The cut-off point of 912 pg/mL had a specificity of 88.9% 95% CI (78.8-94.5%).

NT-proBNP may be useful as a biomarker for paroxysmal atrial fibrillation in ischemic stroke and therefore helpful to reclassify strokes initially categorized as cryptogenic.

Resumo

Mesmo após uma extensa investigação etiologica, em aproximadamente um terço dos acidentes vasculares cerebrais (AVC) isquemicos não é possibel estabelecer uma causa. Uma fracção destes AVCs pode ter sido causada por episodios de fibrilhação auricular paroxistica que não foram identificado. Os estudos mostram que os métodos actuais de diagnostico de fibrilhação auricular paroxistica têm uma baixa sensibilidade. O N-terminal do peptideo natriuretico cerebral (NT-proBNP) pode ser util como biomarcador de AVC cardioembolico.

Os valores séricos de NT-proBNP forem inicialmente determinados em 66 doentes com AVC isquémico. Os doentes com um AVC de etiologica cardioembolica tinham valores de NT-proBNP superiores aos de doentes com uma etiologia não-cardioembolica. O NT-proBNP teve uma boa precisão para diagnosticar AVC cardioembolico relacionado com fibrilhação auricular (AUC-0.92). Dois pontos de corte de valores séricos de NT-proBNP com uma elevada sensibilidade (265.5 pg/mL) e especificidade (912 pg/mL) para o diagnostico de fibrilhação auricular paroxitica foram estabelecidos. Apesar de os valores séricos de NT-proBNP começarem a diminuir 72 horas após o inicio do acidente vascular cerebral, a precisão diagnostica do NT-proBNP para o diagnostico de AVC cardioembolico foi semelhante nas primeiras 72 horas após inicio do AVC. Num estudo de validação realizado em 184 doentes, os pontos de corte de 265.5 pg / mL e 912.0 pg / mL de NT-proBNP tiveram uma sensibilidade, especificidade, valor preditivo positive e valor preditivo no primeiro estudo versus estudo de validação de respectivamente (94.4% vs 100%, 72.9% vs 70.5%, 56.6% vs 59.1%, 97.2% vs 100%) versus (55.5% vs 81.8%, 97.9% vs 87.5, 90.9% vs 73.8%, 83.9 vs 91.9%) para o diagnostico de AVC isquémico de etiologia cardioembolic associado a fibrilhação auricular. Um estudo prospectivo que incluiu 80 doentes foi realizado para avaliar os pontos de corte em doentes com AVC criptogénico.

A área sob a curva ROC da precisão diagnostica dos valores séricos de NT-proBNP para o diagnostico de fibrilhação auricular paroxistica nos doentes com AVC criptogenico foi boa - -

0.83, 95% IC (0.73-0.92). O ponto de corte de 265.5 pg/mL teve uma sensibilidade de 88.2% 95% IC (65.7-96.7%). O ponto de corte de 912 pg/mL teve uma especificidade de 88.9% 95% IC (78.8-94.5%).

O NT-proBNP pode ser util como um biomarcador de fibrilhação auricular paroxística nos doentes com AVC isquémico. Poderá também ajudar a reclassificar AVCs inicialmente categorizados como criptogénico.

Introduction

1.1 Stroke

Stroke is a leading cause of disability and mortality. It is the third cause of death worldwide, the first cause of acquired disability, the second cause of dementia and is associated to significant direct and indirect costs [1]. In Portugal, it has an estimated incidence of 187 per 100000 inhabitants and it is the first cause of death and dependency in patients older than 65 years. A cross-sectional study of a representative sample of the Portuguese population including subjects aged 40 reported a prevalence of atrial fibrillation of 2.5% (2.2-2.8%: 95% CI) [2]. With growing ageing of the world population and increasing incidence of vascular risk factors, the prevalence of stroke-related burden is expected to increase over the next two decades. Although there has been in the last decade an increased use of treatment during ischemic acute stroke, such as thrombolysis (intravenous or intraarterial) and implementation of stroke unit care, these are poorly effective and are available to only a small portion of the population. Resources should be potentiated to improve primary and secondary prevention of stroke. The majority of strokes worldwide (87%) are ischemic. After the diagnosis of stroke, a thorough investigation should be undertaken in order to identify a specific etiology. The identification of a specific etiology has important clinical implications in terms of prognosis, recurrence risk and influences the short term management and the prescription of secondary prevention interventions.

Ischemic stroke is often classified regarding etiology into cardioembolic, large vessel, small vessel, rare causes and cryptogenic causes. It is estimate from large studies that the cause of ischemic stroke is cardioembolic in 17-46% of cases [3].

A cardioembolic etiology is attributable when the embolus that causes the occlusion of a cerebral vessel has a cardiac origin or when there is the passage of embolic material from the venous system through the heart (paradoxical embolism).

Currently the most used ischemic stroke etiology classification is the “Trial of Org 10172 in Acute Stroke Treatment” (TOAST) criteria that first appeared in 1993 [4]. Using this

classification, in order to be classified as having a cardioembolic stroke etiology, patients have to have an arterial occlusions presumably due to an embolus arising in the heart. Cardiac sources are divided into high-risk and medium-risk groups based on the evidence of their relative propensities for embolism. At least one cardiac source for an embolus must be identified for a possible or probable diagnosis of cardioembolic stroke. Evidence of a previous transient ischemic attack or stroke in more than one vascular territory or systemic embolism supports a clinical diagnosis of cardiogenic stroke.

In the A-S-C-O (Phenotypic) classification [5], which was defined in 2009, patients are evaluated for 4 predefined phenotypes, atherosclerosis (A), small vessel disease (S), cardiac disease (C) and other causes (O). The first step is to 'grade' every patient in each of the 4 main

ischemic groups. Three grades of likelihood are considered:

• Grade 1: definitely a potential cause of the index stroke;

• Grade 2: causality uncertain;

• Grade 3: unlikely a direct cause of the index stroke (but disease is present).

Three levels are added for the diagnostic instruments used:

• Level A: direct demonstration by gold-standard diagnostic tests or criteria;

• Level B: indirect evidence or less sensitive or specific tests or criteria;

• Level C: weak evidence in the absence of specific tests or criteria.

The cardiac pathologies that may cause brain embolism may be systematized in seven main groups [6]:

a) Arrhythmias, especially atrial fibrillation (AF), atrial flutter and sick sinus disease;

b) Valve heart diseases including mitral stenosis, prosthetic heart valves, infectious endocarditis and marantic endocarditis;

c) Changes in ventricular myocardium leading to ventricular dilatation, these may can be caused by cardiomyopathies, ischemic heart disease or myocarditis;

d) Intracardiac masses, such as thrombus or tumoral masses, namely atrial myxomas;

e) Intracardiac shunts, specially “patent foramen ovale” and interatrial septal defects, leading to a paradoxical embolism mechanism;

f) Atrial lesions such as left atria enlargement;

g) Aortic lesions, namely aortic atherosclerotic plaques;

The most frequent cardiac source of embolism is the left atrium and the left appendage.

The most common underlying disorder responsible for cardioembolism in ischemic stroke in developed countries is non-valvular atrial fibrillation [3,7].

1.2 Atrial fibrillation

Atrial fibrillation is a supraventricular arrhythmia characterized by an unorganized activation of the electrical atrial activity leading to an inefficient atrial contraction [8].

There are two mechanisms that have been proposed to underlie the initiation and perpetuation of atrial fibrillation: first, rapid ectopic activity may trigger and maintain atrial fibrillation. Second, sustained atrial fibrillation may depend on single or multiple electrical re-entrant circuits resulting from shortening of effective refractory periods and from localized deceleration of intra-atrial conduction. In addition to initiation by electrical trigger beats, re-entry requires a susceptible substrate such as fibrosis. Both mechanisms are not mutually exclusive [9]. There

are also genetic mutations as well as several loci associated with atrial fibrillation that suggest increased individual susceptibility to this arrhythmia [10]. These were identified through analysis of rare monogenetic hereditary atrial fibrillation conditions or using genome-wide association studies. The resulting decrease in atrial contraction leads to blood stasis and to an increased probability of thrombus formation. In locations with stasis, a low laminar blood flux and other factors activate the classical coagulation cascade, leading to thrombus formation. In patients with atrial fibrillation without valve disease, stasis takes place mainly in the atrial appendages. More than 90% of thrombi detected in transesophageal echocardiograms in patients with non-valvular atrial fibrillation were located in the left atrium [11]. It has also been suggested that in patients with atrial fibrillation there is an increase in platelet activation and in thrombin activation that may contribute to a hypercoagulability state [12]. Various inflammatory markers (C-reactive protein, tumor necrosis factor-α, interleukin-2, interleukin-6, and interleukin-8) have been associated to atrial fibrillation. Proposed mechanisms linking inflammation and the prothrombotic atrial fibrillation state include endothelial activation/damage, production of tissue factor from monocytes, increased platelet activation, and increased expression of fibrinogen [13].

Atrial fibrillation has a prevalence of 0.4 to 1% in the population of developed countries in general. The prevalence of atrial fibrillation increases with age, and is higher in men than in women [7,14]. The percentage of strokes attributable to atrial fibrillation increases from 1.5% at 50 to 59 years of age to 23.5% at 80 to 89 years of age [15]. About 70% of patients with atrial fibrillation have an age comprised between 65 and 85 years old [16]. Atrial fibrillation is an established risk factor for ischemic stroke and therefore one of the most important findings in the context of the cardiac investigation of patients with ischemic stroke.

Patients with atrial fibrillation have an associated five-fold increased risk of stroke than patients without this arrhythmia [17]. Stroke related to atrial fibrillation is generally severe with an estimated mortality of 50% in the first year after stroke, has a high recurrence rate and results in significant costs, mobility and mortality [8]. No strategy to pursue a normal sinus rhythm after

atrial fibrillation, including cardioversion, antiarrhythmic drugs or ablation, has been shown to reduce the risk of stroke. In the study ATHENA (A Placebo-Controlled, Double-Blind, Parallel Arm Trial to Assess the Efficacy of Dronedarone 400 mg bid for the Prevention of Cardiovascular Hospitalization or Death from Any Cause in Patients with Atrial Fibrillation/Atrial Flutter), the maintenance of a sinus rhythm following atrial fibrillation by Dronedarone resulted in a decrease in hospitalization by cardiovascular causes in which stroke or transient ischemic attack (TIA) were included [18].

Regarding its temporal pattern, atrial fibrillation can be classified as paroxysmal, persistent or permanent. It is classified as paroxysmal whenever it terminates spontaneously or by an induced mechanism in less than seven days after its onset. Paroxysmal atrial fibrillation may present as a brief single episode of arrhythmia or as clusters of abnormal rhythm of variable duration. It usually lasts less than 48 hours. Whenever atrial fibrillation lasts more than seven days but is ended by a spontaneous or induced way it is classified as persistent. Whenever atrial fibrillation is maintained, regardless of attempted cardioversion or not, it is designated as permanent [8]. Paroxysmal atrial fibrillation comprises between 25% to 62% of atrial fibrillation cases. The natural history is for paroxysmal episodes to increase until they become persistent. Paroxysmal atrial fibrillation has the same risk as persistent or permanent atrial fibrillation to cause an ischemic stroke [19]. It was generally considered that only episodes of paroxysmal atrial fibrillation with more than 30 seconds of duration were of prognostic importance. However, a recent study showed that even high atrial rates of short duration comprising a few seconds are associated with higher risk for acute and chronic brain infarcts [20,21].

Previously, the main cause of atrial fibrillation was rheumatic fever. Currently, due to a decrease in the incidence of rheumatic fever, atrial fibrillation is mainly related to ischemic heart disease, hypertensive heart disease, congestive heart failure and diabetes mellitus. When atrial fibrillation is related to one of these causes it is designated as non-rheumatic or non-valvular atrial fibrillation [8].

Although it is frequently related to structural heart disease, about 45% of patients with paroxysmal atrial fibrillation and 25% with persistent atrial fibrillation have no echocardiographic detectable heart disease [8]. In elderly patients the development of atrial fibrillation is mostly related to cardiac disorders, whereas younger patients may develop atrial fibrillation in the absence of underlying heart disease ("lone AF") [9]. Lone AF usually becomes manifest earlier in life and may have a stronger genetic predisposition than common atrial fibrillation [22].

There are echocardiograph changes that indicate a particular high risk of embolism in patients with atrial fibrillation such as: increased left atria size, decreased flux velocities in the left atrial appendage, mitral valve annulus calcification and left ventricular dysfunction [23]. About 13% of patients with non-rheumatic atrial fibrillation have detectable thrombus in a transesophageal echocardiogram [24]. It is unknown if these patients have a definitely higher risk of ischemic stroke than patients with no detectable thrombus [25].

Some clinical classification schemes have been proposed for predicting stroke in patients with non-valvular atrial fibrillation, one of the most frequently used in clinical practice is the CHADS2 score (Congestive Heart Failure, Hypertension, Age, Diabetes Mellitus and Stroke). This score was proposed in 2006 assigns 1 point for the presence of each of the included risk factors for stroke except for a history of stroke or transient ischemic attack that is assigned with a 2 points score. Patients with a CHADS2 score of 0 or 1 have a 1% annual risk of stroke. CHADS2 scores of 2 have a an annual risk of stroke of 2.5% and patients with a score equal or higher than 3 have an annual risk of stroke of more than 5%. [26]. Since 2006, stronger evidence has accumulated that additional risk factors should be considered in assessing thromboembolic risk and would be of value in identifying those patients at truly low risk.

The additional risk factors have been expressed in the CHA2DS2-VASc (Congestive heart failure, Hypertension, Age≥75 years, Diabetes mellitus, previous Stroke/transient ischemic attack, Vascular disease, Age 65-74 years, Sex category. In this score, age≥75 years and previous stroke carry doubled risk weight [27]. The diagnosis of a cardioembolic etiology

related to atrial fibrillation is particularly important for secondary stroke prevention. Although the majority of patients after an ischemic stroke receive antiplatelets as a secondary prevention treatment, including patients with a stroke of undetermined etiology, in the case of ischemic stroke related to atrial fibrillation, Vitamin-K antagonists are clearly superior to aspirin in the secondary prevention of stroke as shown in the European Atrial Fibrillation (EAFT) trial [28, 29]. The new anticoagulant drugs such as Rivaroxaban [30], Dabigatran [31] or Apixaban [32, 33] also reduce the risk of ischemic stroke in patients with atrial fibrillation. Currently, oral anticoagulation with vitamin K antagonists (INR 2.0-3.0) after ischemic stroke in patients with atrial fibrillation is recommend by the European Stroke Organization (ESO) with an evidence level of Class I, Level A [34].

As ischemic stroke in patents with atrial fibrillation is associated with greater disability and mortality than in those without atrial fibrillation, establishing the presence of underlying atrial fibrillation is of clinical importance.

1.3 Atrial fibrillation diagnosis

The gold standard for the diagnosis of atrial fibrillation is the visual inspection of the electrocardiogram. Guidelines from the European Society of Cardiology (ESC) define atrial fibrillation as a cardiac arrhythmia with the following characteristics: the surface ECG shows absolutely irregular RR intervals; there are no distinct P waves on the surface ECG; and the atrial cycle length (ie, the interval between two atrial activations), when visible, is usually variable and less than 200 ms (>300 beats per min) [35]. Due to the importance of the diagnosis of atrial fibrillation in the context of ischemic stroke all patients should do at least one 12 lead ECG in the etiological workup of stroke, as recommended by both the American Heart Association and the European Stroke Organization [34, 36]. The diagnosis of persistent or permanent atrial fibrillation is straightforward. However, the diagnosis of paroxysmal atrial fibrillation can be quite challenging as it is often asymptomatic and can be present for only a

few fractions of seconds making it frequently clinically underdetected. In one study, less than one-third (32%) of the palpitation symptoms corresponded to atrial fibrillation, with a greater percentage (39%) being in sinus rhythm [37]. This difficulty in diagnosis paroxysmal atrial fibrillation prevents an early detection and timely beginning of therapeutics [38]. In patients with an undetermined stroke etiology and when a cardioembolic stroke etiology is suspected, it is also recommended to do a 24 hours ECG recording (Holter) [34, 36].

To date, several studies have explored the use of prolonged noninvasive and invasive cardiac monitoring devices to identify atrial fibrillation. However, studies show that the current available methods to detect paroxysmal atrial fibrillation have a low sensitivity. It remains elusive which is the best method and the duration of monitoring to detect paroxysmal atrial fibrillation. It is known that a 24 hours ECG recording has a higher sensitivity for the diagnosis of paroxysmal atrial fibrillation than a routine 12 lead ECG. In a systematic review [39] the detection rate of paroxysmal atrial fibrillation by a 24-72 hours ECG recording was of 4.6%. Due to the low detection rate of this examination, there is some controversy regarding its clinical utility and its routine use in the etiological investigation of ischemic stroke [40]. Serial ECG assessments within the first 72 hours of an acute stroke significantly improve detection of atrial fibrillation [41]. Automated analysis of continuous stroke unit ECG monitoring improves paroxysmal atrial fibrillation detection in patients with stroke on stroke units compared with 24-hour Holter ECG [42].

Longer recordings such as with a 7-day ambulatory ECG monitoring using an event-loop recording (ELR) device detected paroxysmal atrial fibrillation in more 6 to 8% of patients after a non-diagnostic 24 hours ECG monitoring [43]. The use of 7 days event-loop recorders at 0, 3 and 6 months after stroke onset detected paroxysmal atrial fibrillation in 14% of patients with an initial negative 24 hours ECG monitoring [39]. Nevertheless in order to register events using this device it is necessary to have the patient collaboration. The patient activates the registry of events after feeling palpitations. Periods of asymptomatic atrial fibrillation are therefore not registered. A Mobile Cardiac Outpatient Telemetry during a period of 21 days in patients with

cryptogenic transient ischemic attack or stroke resulted in a paroxysmal atrial fibrillation detection rate of 23% [44]. One study suggested that in patients with acute ischemic stroke, frequent atrial premature beats (> or = 70/24 hours) could be a marker for individuals who are at higher risk to develop or have paroxysmal atrial fibrillation. For such patients, it was proposed a diagnostic workup with repeated prolonged ECG monitoring to diagnose paroxysmal atrial fibrillation [45]. However the use of long term monitoring was reported to have compliance problems to be cumbersome by both patients and nursing staff [46].

An implantable loop recorder study found paroxysmal atrial fibrillation is 25% of patients with an initial cryptogenic stroke [47]. However, a major limitation of this method is the need of a surgical procedure required for the device implantation.

Table 1 summarizes the main methods of cardiac monitoring.

Methods of cardiac monitoring	pAF detection rate
Non-invasive	
Continuous hospital telemetry Ambulatory ECG (Holter) Patient-triggered event recorder Prolonged ambulatory ECG (mobile cardiovascular telemetry)	8.3%4.6% 6-14% 23%
Invasive	
Implantable loop recorder Pacemakers and defibrillators	25%

Table 1 – Detection rates of paroxysmal atrial fibrillation (pAF) of different methods of cardiac monitoring

There is currently undergoing a clinical trial “Cryptogenic Stroke and underlying Atrial Fibrillation” (CRYSTAL AF), whose goal is to evaluate the incidence of atrial fibrillation and time to atrial fibrillation detection in patients with cryptogenic stroke using an insertable cardiac monitor during a 1-year period [48].

Currently there is no evidence of prognostic utility in ischemic stroke of electrophysiological studies that measure the atrial refractory period and the times of conduction to define an index of atrial vulnerability to fibrillation (latent atrial vulnerability). In the epidemiological study “Ischemic stroke and atrial vulnerability” (ISAV), the presence of atrial vulnerability in patients with cryptogenic ischemic stroke did not correlate with the occurrence of events such as recurrent stroke or atrial arrhythmia [49].

As patients with paroxysmal atrial fibrillation have a high risk of recurrent stroke and anticoagulation is significantly superior to antiplatelets for the secondary prevention of cardioembolic stroke, alternative ways to detect a possible cardioembolic etiology in cryptogenic stroke must be considered.

Currently, about one third of ischemic strokes are classified as of undetermined etiology. It is possible that a fraction of these strokes named as cryptogenic may be due to an undetected episode of atrial fibrillation [50, 51].

The importance of a correct identification of stroke etiology leads me to posit that biomarkers studied acutely in patients with brain ischemia could identify some cardioembolic sources of embolism.

1.4 Biomarkers

The first reference to the term biomarker appeared in PubMed in 1977 [52]. It was first defined in 1989 in the Medical Subject Heading (MeSH) as a “ Measurable and quantifiable biological

parameters (e.g., specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health and physiology related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, pregnancy, cell line development, epidemiologic studies, etc." [53]. In 2008, the definition was reviewed and biomarker became defined as "a molecular, biological, or physical characteristic that indicates a specific physiologic state. It is used in clinical practice to identify risk for disease, diagnose disease and its severity, guide intervention strategies, and monitor patient responses to therapy" [54].

Biomarkers have been progressively recognized as important diagnostic tools. Ideally, a biomarker should be highly sensitive, specific, accessible, accurate, reproducible by an analytical method, cost-effective and have a result that can be easily interpreted by a physician [55].

Examples of blood biomarkers used in clinical practice include cardiac troponins in myocardial infarction, human choriogonadotropin to diagnose pregnancy, and creatinine that is used to monitor renal function.

In the context of stroke, biomarkers have been studied for [56]:

- Diagnosis of ischemic stroke;
- Diagnosis of ischemic stroke versus hemorrhagic stroke;
- Identification of ischemic stroke phases (as markers of definitive ischemic lesion or of potentially recoverable tissue corresponding to penumbra);
- Determination of stroke etiology;
- Determination of clinical prognosis;
- Response to treatment

Biomarkers under investigation in stroke are or specific to the central nervous system or markers of systemic inflammation, fibrinolysis or hemostasis. Possible biomarkers from the blood, cerebrospinal fluid and brain tissue have been investigated [57]. Preferably a blood or serum derived biomarker should be used, as it is more accessible, less invasive and could be monitored in time through serial measurements.

There are two great obstacles to the use of biomarkers in cerebrovascular pathology:

a) The presence of the blood-brain barrier that difficult and delays the release of proteins of neuronal or glial origin into the bloodstream after stroke;

b) Many of the potential serum biomarkers of cerebral ischemia and inflammation have a low specificity and may also be increased in situations that can be confounded with stroke in their presentation such as acute myocardial infarction or central nervous system inflammation [58]

Presently around 58 possible stroke related biomarkers have been studied. Four of these proteins have been studied as possible blood based biomarkers of cardioembolic stroke. There are also studies investigating if there are gene expressions signatures in blood that could be suggestive of cardioembolic stroke [59,60]. Using whole-genome microarrays, a 40-gene profile that distinguished cardioembolic stroke from large-vessel stroke, and a separate 37-gene profile that distinguished cardioembolic stroke due to atrial fibrillation from nonatrial fibrillation causes was identified. These genes play roles in inflammation [59]. Nevertheless none of these biomarkers can be recommend to be used in clinical practice.

Table 2 displays the characteristics of the main serum biomarkers that have been evaluated in the context of ischemic stroke related to atrial fibrillation.

Biomarker	General characteristics	Study results
Brain Natriuretic Peptide and NT-proBNP [61]	Peptide with heart and brain production	↑acute ischemic stroke ↑cardioembolic stroke
D-dimers [61,62,63]	Product of fibrin degradation	↑acute, subacute and chronic phases of ischemic stroke ↑cardioembolic stroke
Von Willebrand factor	Glycoprotein involved in hemostasis	↑ acute ischemic stroke
Soluble Thrombomodulin [64]	Integral membrane protein expressed on the surface of endothelial cells, serves as a cofactor for thrombin	↑ acute ischemic stroke (atrial fibrillation)

Table 2 – Resume of the characteristics of the main serum biomarkers that have been studied in the context of ischemic stroke related to atrial fibrillation, ↑ - increased

One of the substances that have been studied as a possible biomarker of cardioembolic stroke is the N-terminal of the brain natriuretic peptide (NT-proBNP).

1.5 Brain natriuretic peptide and N-terminal proBrain natriuretic peptide

NT-pro BNP is part of a group of natriuretic peptides, phylogenetically conserved along time that includes peptides such as atrial natriuretic peptide (ANP), natriuretic peptide type C, urodilatin and the *Dendroaspis* natriuretic peptide [65].

NT-proBNP is coded by a gene composed by three exons and two introns that is located in chromosome 1p36.2. It is initially produced as a prepropeptide of 143 amino acids. This prepropeptide is proteolytic cleaved into a non-active N-terminal fragment composed by 108 amino acids designated proBNP. ProBNP after secretion is divided in two fractions by two proteolytic enzymes. These two fractions are BNP that is biological active (aa 77-108) and the N-terminal-proBNP (NT-proBNP) (aa 1-76) without biological activity [66]. BNP and NT-proBNP concentrations can be determined in blood samples [67]. The half-life of these two peptides differs. NT-proBNP has a half-life superior to BNP. NT-proBNP has a half-life of 120 minutes and BNP a half-life of 22 minutes [68]. It is due to this difference in half-life that most essays measure NT-proBNP instead of BNP.

Although NT-proBNP was initially isolated in porcine brain in 1988 [69], explaining its designation as brain natriuretic peptide, subsequent experiences showed that it also had a cardiac production [70]. Currently it is considered that the heart is its main production site. In the brain, NT-proBNP is mainly produced in the hypothalamus. The cerebral cortex, thalamus, pons have also been pointed as possible production sites [71]. NT-proBNP in normal conditions does not cross the bold-brain barrier [72].

In the heart, BNP can be produced by both atria and ventricles [72]. After being produced NT-proBNP and BNP are mainly secret in “bursts”. Their storage in granules is minimal [73].

Concerning tissue expression, BNP seems to be more present in the atria rather than in the ventricles. However, due to the larger ventricular mass, 70% of all BNP is produced by the

ventricle in normal conditions [74]. The observation of ventricular BNP gene expression in ventricular disease may have contributed to the common notion that BNP is mainly a ventricular hormone.

The main stimulus for the synthesis and excretion of BNP is myocites stretching, mainly in the context of volume [75] or cardiac pressure overload [76]. After myocites stretching there is a rapid activation, within hours, of NT-ptoBNP gene expression [77]. Heart fibroblasts can also produce NT-proBNP. Heart hypertrophy, fibrosis and hypoxia can also stimulate NT-proBNP production [78]. Certain hormones (catecholamines, angiotensin II and endothelin-1) may stimulate the production of NT-proBNP through paracrine or endocrine mechanisms [79].

Biological actions of BNP are mediated by the receptor of the natriuretic peptides of type A (NPR-A). This receptor is a guanylyl cyclase receptor. It is a transmembrane receptor with 120 kD. It is composed by an extracellular region that connects to BNP and an intracellular kinase and guanylyl cyclase domain with enzymatic activity. This receptor is mainly located in the cellular membrane of the endothelium of small vessels [80]. BNP connection to the receptor leads to the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) [81]. A kinase protein dependent on cGMP (PKG or cGK) is the principal intracellular mediator of cGMP signals through a catalytic transference of the phosphate of present in TPA to a serine or threonine residue present in the target protein [82]. Signal transduction activated by these receptors is terminated by a GMP phosphodiesterase that modulates the intracellular concentration of cGMP and the duration and magnitude of the response [83].

BNP acts by an endocrine mechanism. It has an important role in the cardiovascular homeostatic regulation and in volume control. BNP biological effects include natriuresis, diuresis, vasodilatation, inhibition of the renin-angiotensin-aldosterone axis and of the sympathetic nervous system [84]. In the heart BNP has a lusitropic and antifibrotic effect [85].

Know mechanisms of BNP inactivation include connection to the type C receptor of clearance of the natriuretic peptides and proteolysis by the peptidase NEP 24.1. NT-proBNP is cleared from circulation through the kidney by direct filtration and passive excretion [86].

In healthy individuals, BNP and NT-proBNP plasmatic concentrations are similar. They can be detected in venous blood samples in picomolar concentrations. However, in congestive heart failure patients, NT-proBNP concentration is two to ten times higher than BNP concentration. The explanation for this difference is unknown. Serum NT-proBNP and BNP levels in healthy individuals increase with age and are higher in women than in men [87]. The reason for this age-related increase in BNP or NT-proBNP is presumed to be related to the parallel age increase in subclinical structural heart disease, including heart muscle disease, diastolic abnormalities, valve disease and arrhythmia [88]. An age-related decrease in renal function is also considered to be partially responsible. Ninety percent of young adults have NT-proBNP levels below 70 pg/mL [89]. NT-proBNP plasmatic levels correlate with left ventricular mass. A NT-proBNP cut-off superior to 300 pg/mL has been suggested for the diagnosis of congestive heart failure [90]. NT-proBNP is currently used as a marker of left ventricular dysfunction and of prognosis in patients with congestive heart failure and in acute ischemic heart disease [91]. High NT-proBNP levels have been registered in patients with renal failure, anaemia, acute pulmonary embolism, pulmonary hypertension, sepsis, hyperthyroidism, mitral and aortic regurgitation and dysrhythmias such as atrial fibrillation [92, 93].

Patient with lone paroxysmal atrial fibrillation seem to have increased NT-proBNP levels when compared to patients in sinus rhythm, in the absence of a structural heart disease [94]. Increased plasmatic levels of NT-proBNP have been found in patients with permanent or paroxysmal atrial fibrillation with preserved left ventricular systolic function [95]. One study showed that in 81% of patients NT-proBNP levels increased within four hours of the onset of atrial fibrillation [96]. It has also been documented, after electric cardioversion of atrial fibrillation to sinus rhythm, a return to baseline of NT-proBNP values [97]. NT-proBNP has been shown to be a remarkable predictor of incident atrial fibrillation in the general population, independently of

any other previously described risk factor [98]. Recent studies that suggest that NT-proBNP is a predictor of atrial fibrillation following cardiac surgery [99-101] and successful cardioversion [102].

There is currently no established explanation for the increase of BNP in the context of atrial fibrillation. In the isolated atria tissue of rat, alpha1-adrenergic stimulation with phenylephrine induces genetic expression of BNP [103]. It is unknown if alpha1-adrenergic stimulation results in an increase in the synthesis of BNP in patients with atrial fibrillation. A study that analysed human right atrial tissue found that persistent atrial fibrillation was associated to a high expression of proBNP mRNA. However, patients with paroxysmal atrial fibrillation did not have changes in proBNP gene expression [104].

1.6 NT-proBNP and BNP in ischemic stroke

Although NT-proBNP presence was first described in the brain tissue, information regarding NT-proBNP in ischemic stroke is small.

Some studies showed an acute increase in NT-proBNP and BNP serum levels in acute ischemic stroke (Table 3). These studies initially aimed to determine if NT-proBNP or BNP could be used as prognostic markers of acute ischemic stroke along with other markers of myocardial injury such like troponins. It was suggested that it could be used as a marker of long term bad prognosis. It was then noticed that patients with acute ischemic stroke tended to have higher levels of NT-proBNP and BNP when taking in account the cut-off points used for the diagnosis of congestive heart failure.

Four possible explanations for the increase of NT-proBNP and BNP during acute ischemic stroke where proposed in these studies:

- Patients with acute ischemic stroke frequently have several vascular risk factors and may have an associated acute or chronic heart failure. Therefore, the increase in NT-proBNP levels could be due to ventricular dysfunction;

- The increase in NT-proBNP levels could be due to atrial fibrillation. Atrial fibrillation is a major risk factor for ischemic stroke and a known cause of NT-proBNP increase;

- As the brain is a site of BNP and NT-proBNP production, although it only produces a small amount of these peptides it is possible that after an acute ischemic lesion there is a release of BNP and NT-proBNP that is measurable in the plasma.

- BNP has an inhibitory action in the central and peripheral sympathetic nervous system. BNP could be increased in acute ischemic stroke as a response to the increase sympathetic activity that occurs after stroke

- Autonomic changes of acute ischemic stroke are frequently associated to right insular cortical ischemia [105]. Insular cortical ischemia has been associated to an increase in the serum levels of norepinephrine, electrocardiographic changes and changes in the circadian patterns of blood pressure. In these cases, there is an inflammatory response with increase of interleukin 6. Interleukin 6 can directly increase BNP and NT-proBNP levels.

Study	Nº of patients	Results
Etgen, 2005 [106]	174	Increase of NT-proBNP levels in acute ischemic stroke, when using as cut-off points the levels used for the diagnosis of congestive heart failure Increase of NT-proBNP levels could be due to ventricular

		dysfunction or be due to sympathetic nervous system activation
Makikallio, 2005 [107]	51	Increase of NT-proBNP levels in acute ischemic stroke when comparing with healthy controls. Increase of NT-proBNP could have a brain origin
Nakagawa, 2005 [108]	88	Increase of NT-proBNP in the acute phase of ischemic stroke, decrease in the subacute phase NT-proBNP levels correlated with mean blood pressure
Giannakoulas, 2005 [109]	30	Higher increase of NT-proBNP in cardioembolic stroke than in large vessels diseases related ischemic stroke Increase of NT-proBNP due to brain ischemia or direct myocardial dysfunction
Jensen 2006 [110]	250	NT-proBNP levels in the second day after ischemic stroke associated to 6 months mortality
Itumur 2006 [111]	57	Patients with ischemic stroke with insular cortical involvement and with signs of myocardial ischemia had higher levels of NT-proBNP than other patients with ischemic stroke. However this increase was not statically significant
Yip 2006 [112]	86	Ischemic stroke patients with higher NT-proBNP levels had a higher number of unfavorable clinical events (acute myocardial infarction, congestive heart failure, recurrent stroke or any other cause of death)
Koenig 2007	72	The increase of NT-proBNP in acute ischemic stroke was not

[113]		independently associated to congestive heart failure
Di Angelantonio 2007 [114]	48	BNP is a marker of left atrial dysfunction even in the absence of atrial fibrillation
Montaner 2008 [61]	707	Several potential biomarkers were studied. Levels of BNP> 76 pg/mL in patients with acute ischemic stroke indicated a cardioembolic etiology with a sensitivity of 72%, a specificity of 69% and a negative predictive value of 82%.
Naya 2008 [115]	76	NT-proBNP levels and the evaluation of flow in the left atria appendage could help to distinguish cardioembolic stroke related to atrial fibrillation from other etiologies
Gartner 2008 [116]	222	A statistically significant association was found between the plasmatic concentration of BNP and the severity of clinically silent brain infarctions related to atrial fibrillation
Tomita 2008 [117]	79	Plasmatic levels of BNP were statistically significantly higher in patients with acute ischemic stroke with a large vessels etiology. Patients with a cardioembolic etiology were excluded from the study. BNP levels were positively correlated with the NIH stroke scale classification and with the volume of the ischemic lesion
Shibazaki K, 2009 [118]	200	Plasma BNP level is significantly higher in cardioembolic patients than in other stroke subtypes, and thus physicians should strongly consider cardioembolism when the plasma BNP level is over 140.0 pg/mL in patients with acute ischemic stroke

Saritas A 2010 [119]	123	Patients who had atrial fibrillation in their electrocardiography had significantly higher BNP levels than patients in sinus rhythm. A positive correlation was found between plasma BNP levels with age, blood urea nitrogen and NIHSS and a negative correlation was found between plasma BNP levels and GCS. There was a significant difference between the BNP levels of NIHSS groups.
Okada 2010 [120]	237	High plasma BNP level should be a strong predictor of delayed AF after ischemic stroke or TIA
Kimura 2010 [121]	79	The combination of AF and BNP>150pg/ml was a useful predictor for no early recanalization after IV-t-PA therapy
Okada 2011 [122]	67	In patients with AF in acute ischemic stroke or TIA , a BNP concentration of>140.0 pg/ml (odds ratio, 5.62; 95% CI, 1.39-22.66, p = 0.015) was an independent factor associated with left atrial thrombus.
Shibazaki 2011 [123]	221	Plasma BNP level of > 320 pg/ml (OR, 4.74; 95% CI, 1.260-17.800, P = 0.0213) were independent factors associated with in-hospital death.

Table 3- Previous studies reporting NT-proBNP or BNP changes in patients with acute ischemic stroke

In a previous study [124], we analyzed whether NT-proBNP was elevated in patients with acute ischemic stroke of cardiac cause. We used a sample of consecutive acute stroke patients with ischemic or intracerebral hemorrhage that were admitted from November 2007 to August 2008 to the Stroke Unit of Hospital de Santa Maria, Lisbon, Portugal.

Patients with a history of conditions known to increase NT-proBNP (acute ischemic heart disease, heart failure, heart valve disease, renal failure, cardiomyopathies, pulmonary hypertension, anemia) were excluded. Patients with subarachnoid hemorrhage, cerebral venous thrombosis and transient ischemic attack were also not included in this study.

After patient admission from the emergency department, information on demography, vascular risk factors, or previous atrial fibrillation was collected. A brain CT scan, done after hospital admission, was used to classify strokes as hemorrhagic or ischemic. It was repeated 48–72 h after hospital admission to outline the ischemic area. When the ischemic area was not evident in a CT scan, a 1.5 T magnetic resonance imaging (MRI; including diffusion-weighted imaging) was performed. All patients had transcranial Doppler and carotid and vertebral duplex scanning. Etiological workup included, in all patients with ischemic stroke, complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis and coagulation studies. In patients less than 55 years old, workup included autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antitrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies. Transthoracic echocardiogram was performed in all patients. Patients with echocardiographic evidence of heart disease (shortening fraction less than 30%, valve dysfunction, cardiomyopathies, akinetic ventricular wall regions) were excluded.

The following parameters were registered:

- left atria autocontrast
- intracardiac thrombus
- shortening fraction
- patent foramen oval (PFO), and

- akinetic ventricular wall regions.

In patients less than 55 years old a transesophageal echocardiogram was also performed. To determine the presence of atrial fibrillation at least two ECGs were done during hospital stay, and when the stroke cause remained undetermined a 24 h Holter monitoring was also performed. In patients whose stroke cause remained undetermined, lumbar puncture or cerebral angiography was done. Patients with hemorrhagic stroke had blood analysis, ECG and a transthoracic echocardiogram. Ischemic stroke causes were classified according to TOAST [4] classifications in five groups; large-artery atherosclerosis, cardioembolic, small-vessel occlusion, stroke of other determined cause and stroke of undetermined cause. For the present study, patients were subdivided in two groups; cardioembolic cause (corresponding to the cardioembolic classification of TOAST) and noncardioembolic cause (all other four groups of the TOAST classification). Ischemic stroke topography was dichotomized in anterior/carotid territory or posterior/vertebrobasilar territory, accordingly to CT or MRI information. Infarct size was classified according to the ASPECTS scale [125], using CT-scan or MRI information. To evaluate autonomic nervous system activation three measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were done at eight-hour intervals after patient admission. In the first 72 h after symptoms onset, four milliliters of blood was drawn from a peripheral vein. Blood samples were immediately centrifuged at 1600 g during 15 min. Serum concentration of NT-proBNP was determined by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer [126].

A descriptive statistical analysis of demographic and vascular risk factors of patients with ischemic stroke, hemorrhagic stroke, cardioembolic and noncardioembolic cause was performed. Data distribution was evaluated using histograms and a one-sample Kolmogorov–Smirnov test. For comparison between groups the w2-test, Fisher exact test, Mann–Whitney test or t-test were used as appropriate. As NT-proBNP values did not follow a normal distribution, a logarithm transformation was done. To compare NT-proBNP values between different timings of blood collection, a one-way analysis of variance was used. t-test was used to compare mean

values of NT-proBNP between patients with intracerebral hemorrhage versus ischemic stroke , cardioembolic stroke versus noncardioembolic stroke and cardioembolic stroke related to atrial fibrillation versus noncardioembolic stroke. Receiver operating characteristic (ROC) curves were used to test the ability of NT-proBNP to identify cardioembolic stroke and cardioembolic stroke associated with atrial fibrillation. The area under the curve (AUC) for each ROC curve was determined. Based on the ROC curves, NT-proBNP values with the highest sensitivity and specificity for the diagnosis of cardioembolic stroke and cardioembolic stroke related to atrial fibrillation were determined as well as the corresponding positive and negative predictive values (NPV).

To study the association between NT-proBNP and systolic blood pressure, diastolic blood pressure and heart rate, a simple linear regression was calculated. The corresponding regression coefficients with 95% confidence intervals (CI) were determined. To evaluate differences in NT-proBNP values between arterial territories, t-student test was used. To evaluate the association between ischemic area and NT-proBNP value a Kendall correlation analysis was performed.

From November 2007 to August 2008, 202 stroke patients were admitted to the Stroke Unit. Ninety-two patients were included. The main reasons for exclusion were: heart diseases known to increase NT-proBNP (32.7%), subarachnoid hemorrhage (29.1%) and patient admission 72 h after stroke onset (18.2%). Included patients had a mean age of 58.6 (SD +/- 14.4) years. Women were 64.1% of the patients. Sixty-six (71.7%) patients had an ischemic stroke and 26 (28.3%) an intracranial hemorrhage (Table 4).

	Ischemic stroke (n=66)	Hemorrhagic stroke (n=26)	p
Gender, female (%)	26 (39.4)	7(26.9)	0.26
Age, years (mean, SD)	60.6(14.9)	53.6(11.9)	0.035

Vascular risk factors			
Hypertension (%)	33(35.9)	15 (16.3)	0.51
Diabetes mellitus (%)	8 (8.7)	4 (4.3)	0.74
Dyslipidemia (%)	20 (22.0)	5 (5.5)	0.27
Smoking (%)	26 (39.4)	9 (34.6)	0.67
Previous AF (%)	7 (10.6)	0 (0)	
Vital parameters			
SBP, mmHg (mean, SD)	140.4 (23.2)	149.5 (28.0)	0.16
DBP,mmHg (mean, SD)	71 (15)	81 (14)	0.01
HR, bpm (mean, SD)	69 (13)	65 (15)	0.33

Table 4 – Demographic data, vascular risk factors and vital parameters of patients with ischemic and hemorrhagic stroke, SBP – Systolic blood pressure, DBP – diastolic blood pressure, HR - heart rate, SD – standard deviation, AF – atrial fibrillation

According to the TOAST classification 28 patients had a cardioembolic cause and 38 were noncardioembolic: 12 (18.2%) large arteries; seven (10.6%) small vessels; five (7.6%) other determined; 14 (21.2%) undetermined. Cardioembolic cause included 18 patients with atrial fibrillation (12 paroxysmal, six permanent) and 10 patients with patent foramen ovale. Demographic data, vascular risk factors and vital parameters of patients with cardioembolic ischemic stroke and noncardioembolic ischemic stroke were not significantly different (Table 2). The two subgroups of patients with cardioembolic and noncardioembolic cause were not significantly different concerning arterial territory or insular involvement or infarct size evaluated by the ASPECTS scale (Table 5).

	Cardioembolic stroke (n=28)	Noncardioembolic stroke (n=38)	p
Gender, male (%)	15 (53.6)	25 (65.8)	0.32
Age, years (mean, SD)	63 (16)	59 (14)	0.24

Vascular risk factors			
Hypertension (%)	12 (18.2)	21 (31.8)	0.32
Diabetes mellitus (%)	3 (4.5)	5 (7.6)	0.99
Dyslipidemia (%)	8 (12.3)	12 (18.5)	0.74
Smoking (%)	16 (57.1)	10 (26.3)	0.60
Anterior territory (%)	21 (75.0)	27 (77.1)	0.84
Insular involvement (%)	14 (50)	11 (28.9)	0.08
ASPECTS (median)	6	7	0.19
Vital parameters			
SBP, mmHg (mean, SD)	139 (22)	141 (24)	0.68
DBP,mmHg (mean, SD)	68 (12)	73 (16)	0.19
HR, bpm (mean, SD)	70 (16)	68 (11)	0.65

Table 5 – Demographic data, vascular risk factors, stroke characteristics and vital parameters of patients with cardioembolic and noncardioembolic stroke, SBP – Systolic blood pressure, DBP – diastolic blood pressure, HR - heart rate, SD – standard deviation

No intracardiac thrombus or left atria autocontrast were detected during echocardiography. The NT-proBNP values followed a positively skewed distribution and ranged from 8 to 6378 pg/ml with a median of 177.0 pg/ml. After a logarithm transformation, a new variable was obtained, which followed a normal distribution. In 21 patients (22.8%), blood was collected in the first 24 h, in 62 patients (67.4%) in the first 24–48 h and in nine patients (9.8%) in the first 48–72 h. The mean value (95% CI) of NT-proBNP in patients with ischemic stroke was 223.18 (157.42–316.40) pg/ml and in patients with hemorrhagic stroke was 133.34 (74.13–239.82) pg/ml. However, this difference was not statistically significant (P=0.12). The mean of NT-proBNP values in patients with ischemic stroke in the carotid artery territory was 275.88 (95% CI 179.47–419.89) pg/ml and in patients with stroke in the vertebrobasilar territory was 138.83 (74.44– 259.82) pg/ml. This difference was not statistically significant (P=0.10). No statistically

significant association was found between infarct size evaluated by ASPECTS scale and serum values of NT-proBNP (P=0.11). No significant linear relationship was found between NT-proBNP values and systolic blood pressure (P=0.091) or diastolic blood pressure (P=0.26). A significant linear relationship was found between NT-proBNP values and heart rate with a regression coefficient of 0.025 pg/ml/bpm, (P= 0.039). The mean of NT-proBNP values for cardioembolic stroke was significantly higher (P<0.001) (491.6; 95% CI 283.7–852.0 pg/ml) than for noncardioembolic ischemic stroke (124.7; 86.3–180.2 pg/ml) (Figure 1).

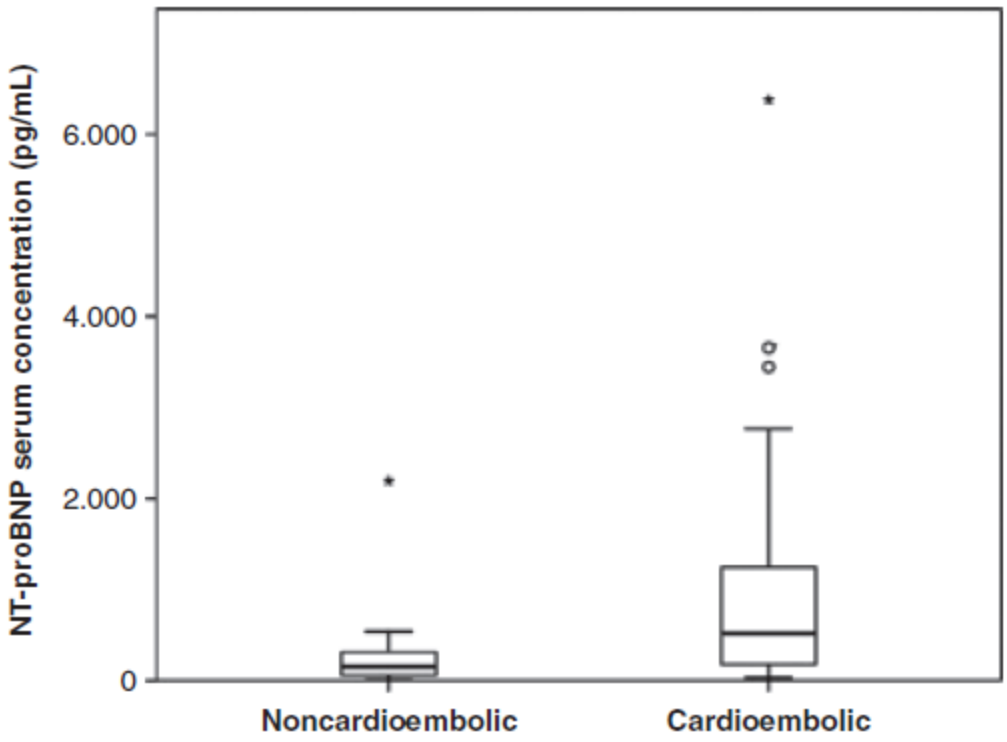

Figure 1- Serum concentration of NT-proBNP in patients with noncardioembolic and cardioembolic ischemic stroke. Boxplots present median values and interquartile ranges

The ROC curve of NT-proBNP values for the diagnosis of cardioembolic stroke had an AUC (95% CI) of 0.77 (0.65–0.89). The cut-off point with the highest sensitivity and specificity was set at 265.5 pg/ml (71.4% and 73.7% respectively). This point had a NPV of 77.8%and a

positive predictive value (PPV) of 66.6% (Figure 2). The AUC of NT-proBNP obtained for the diagnosis of cardioembolic stroke related to AF was 0.92 (0.86–0.99). This AUC value was higher than the value obtained for the diagnosis of cardioembolic stroke in general (0.92 versus. 0.77). After analysis of the ROC curve for the diagnosis of cardioembolic stroke related to atrial fibrillation a cut-off point of 265.50 pg/ml was determined (sensitivity of 94.4%, specificity of 72.9%, positive predictive value of 56.6% and a negative predictive value of 97.2%). This cut-off point had an extremely high negative predictive value. However, in the context of clinical decisions, it is more important to confirm the diagnosis of cardioembolism as it leads to a change in clinical decision; therefore another cut-off point with a higher positive predictive value was determined. The cut-off point of 912.0 pg/ml had a sensitivity of 55.5%, specificity of 97.9%, positive predictive value of 90.9%, and a negative predictive value of 83.9% (Figure 2).

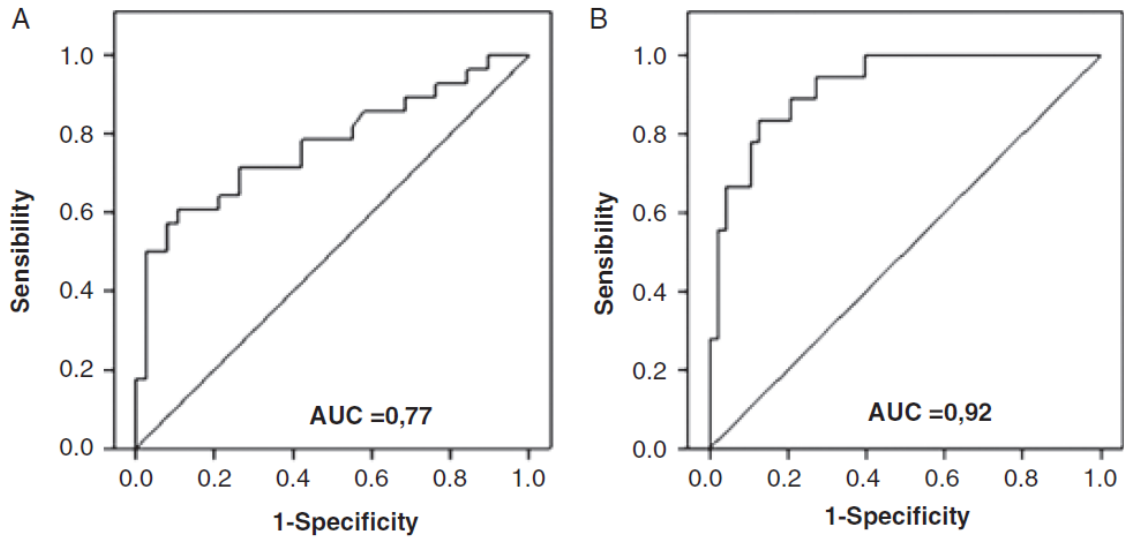

Figure 2 –ROC curves illustrating the accuracy of NT-proBNP to identify cardioembolic stroke (A) and cardioembolic stroke associated to atrial fibrillation (B)

This study suggests that the increase of NT-proBNP which occurs during stroke has a cardiac origin and may be due to atrial fibrillation. The mean value of NT-proBNP in patients with

cardioembolic ischemic stroke was significantly higher than in patients with noncardioembolic ischemic stroke. No significant association was found between stroke territory or infarct size and NT-proBNP values, or between systolic blood pressure or diastolic blood pressure and NT-proBNP values. The ROC curve for the diagnosis of cardioembolic stroke had an AUC of 0.77 which corresponds to a good ability of NT-BNP to diagnose cardioembolic stroke. The ROC curve for the diagnosis of cardioembolic stroke associated to atrial fibrillation had an AUC of 0.92 which suggests that NT-proBNP has a very good ability to diagnose ischemic stroke associated to atrial fibrillation, being useful to differentiate it from other etiologies. Although previous studies had suggested a cardiac cause they had not excluded the presence of confounding variables, which could have caused the increase of NT-proBNP. Namely Montaner [61] and Shibazaki [118] did not exclude the presence of renal failure, heart failure and ischemic heart disease. In our study, to decrease possible confounding factors, patients with known causes of NT-proBNP increase, such as renal failure and heart failure, ischemic heart disease and valvular heart disease, were excluded, using both clinical and echocardiographic evidence. Also, Montaner [61] and Shibazaki [118] did not analyze if this increase could be due to the large infarct area that patients with cardioembolic stroke tend to have [127]. In their patients, the increase of NT-proBNP could have been due to a large infarct area, as it is established that NT-proBNP is also produced in the brain [69, 128]. In our study, we analyzed this variable and we did not find an association between stroke territory or infarct size and NT-proBNP values. One recent study [122] suggested that the increase of NT-proBNP in stroke could be due to atrial fibrillation. However, the authors did not exclude patients with heart failure and found that the variable ‘congestive heart failure’ was significantly higher in the atrial fibrillation group than in non-atrial fibrillation group. Therefore the increase of the NT-proBNP could not be securely attributed to atrial fibrillation, because heart failure is a cause of NT-proBNP increase [91]. In our study, levels of NT-proBNP were compared between hemorrhagic and ischemic stroke based in the hypothesis that if the increase of NT-proBNP was purely due to a ischemic stroke cause subtype – cardioembolic – and not to other factors such as autonomic activation, there

would be higher levels of NT-proBNP in patients with ischemic stroke than in hemorrhagic stroke. In fact, patients with ischemic stroke had higher levels of NT-proBNP (223.18 (157.42–316.40) pg/ml) than patients with hemorrhagic stroke (33.34 (74.13–239.82) pg/ml). However, this difference was not statistically significant (P=0.12) probably due to the modest number of patients included. When comparing only patients with ischemic stroke, after excluding possible confounding variables, we found that the mean value of NT-proBNP in patients with cardioembolic ischemic stroke was significantly higher than in patients with noncardioembolic ischemic stroke. Due to the exclusion criteria, the number of possible heart embolic sources was restricted to two: atrial fibrillation and patent foramen ovale. Patent foramen ovale can be a cardioembolic source due to paradoxical embolism or due to the induction of changes in the electrical activity of the left atria leading to atrial arrhythmias, such as paroxysmal atrial fibrillation or atrial flutter [129]. It may be therefore able to increase NT-proBNP values. After analysis of the ROC curve for ischemic stroke associated to atrial fibrillation, a cut-off point of 265.5 pg/ml with a high sensitivity (94.4%) and a high negative predictive value (97,2%) was determined. The cut-off point of 912.0 pg/ml had a positive predictive value of 90.9%. If this cut-off point is confirmed in another sample it can lead to a high suspicion of atrial fibrillation in patients with stroke of undetermined cause. A NT-proBNP level above this cut-off may help to select patients for prolonged heart rhythm monitoring to detect paroxysmal atrial fibrillation. In the previously mentioned studies [61, 121, 122], blood was drawn in the first 24 h after stroke onset. In our study, blood was drawn in the first 72 h after stroke onset (although mostly in the first 24–48 h). The option for an enlarged inclusion time was based on the knowledge that a sizable proportion of patients does not go to the hospital in the first 24 h after stroke onset [130]. Available data is unclear regarding the timing when maximum serum concentration of NT-proBNP in ischemic stroke is achieved. Giannakoulas [109] did not find a statistically significant difference between day one and six after stroke onset. Jensen [110] noticed a peak in the second day, with a progressive decrease in NT-proBNP until day five. Iltumur [111] described highest values of NT-proBNP in the day of stroke onset. In our study no significant

difference was found between the different timings of drawing blood samples. One limitation of our study is the modest number of patients included; nevertheless it obtained some statistically significant results. Other limitations relate to the use, in the majority of patients, of CT instead of MRI to evaluate the infarct area and location. The study of possible biomarkers of ischemic stroke subtypes may be clinically valuable. Our results suggest that NT-proBNP can be a useful biomarker of certain causes of cardioembolic stroke, namely atrial fibrillation. Our results must be replicated in an independent sample. It is necessary to conduct another study on a different sample, with a larger number of patients to validate the cutoff points established. It is also necessary to identify possible sources of variation of NT-proBNP and in particular to establish the profile of time course of NT-proBNP after an ischemic stroke.

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Hypothesis

The increase of serum NT-proBNP levels in ischemic stroke may be used in the classification of the etiology of ischemic stroke as a marker of high risk cardioembolic source related to atrial fibrillation

Aims

1) Describe the temporal evolution of the values of NT-proBNP in the first 72 hours after onset of symptoms of ischemic stroke, making measurement of the NT-proBNP 24, 48 and 72 hours after onset of stroke symptoms.

2) Validate the previously determined values of sensitivity and specificity for the cutoff points of 265.5 pg / mL and 912.0 pg / mL of NT-proBNP (94.4%, 72.9% and 55.5 %, 97.9%) for the diagnosis of ischemic stroke of cardioembolic etiology associated with atrial fibrillation.

3) To analyze if the previous established cut-off values of NT-proBNP have a similar accuracy to diagnose cryptogenic stroke associated to paroxysmal atrial fibrillation.

4) To analyze if individuals with stroke of undetermined etiology, with a clinical or imaging pattern suggestive of cardioembolism, at the hospital and in whom atrial fibrillation (ECG or Holter) is subsequently detected, have higher values of NT-proBNP at the index event than individuals with stroke of undetermined etiology without these characteristics.

1. Time course of NT-proBNP levels after acute ischemic stroke

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- Fonseca AC, Sampaio Matias J, Pinho e Melo T, Pires C, Canhão P, Geraldes R, J.M. Ferro. Time course of N-terminal pro-brain natriuretic peptide in acute ischemic stroke. Cerebrovasc Dis 2011;31(suppl 2):53.

Introduction

The identification of a specific ischemic stroke etiology has important clinical implications in terms of prognosis, recurrence risk and influences the short term management and the prescription of secondary prevention interventions.

Particularly important is the identification of a cardioembolic etiology, namely atrial fibrillation (AF), as studies have shown that anticoagulation is superior to antiplatelet agents for the prevention of recurrent stroke [1]. Stroke related to AF tends to be severe, with a mortality rate at the first year of 50% and is associated to a high recurrence rate [2].

Identifying paroxysmal AF can be particularly difficult as auxiliary complementary examinations can be entirely unremarkable. Literature shows that the current available methods (ECG, routine telemetry during inpatients admission, Holter monitoring, 30-day event monitoring devices) to detect paroxysmal AF have a low sensitivity [3]. Therefore it is plausible that cases of undetected paroxysmal AF may contribute to a large fraction of the approximately 1/3 of strokes which are currently classified as of undetermined etiology [4].

Serum biomarkers may be useful in this context. Recent studies suggest that NT-proBNP may be useful as a serum biomarker of cardioembolic stroke, namely associated with AF [5,6,7]. NT-proBNP is produced by the atrial and ventricle myocytes in stressful conditions such as hemodynamic overload. Namely, following the hemodynamic effect of atrial fibrillation, granules stored in atrial myocites are secreted as BNP and NT-proBNP [8], leading to the high levels of NT-proBNP.

However, to use NT-proBNP as a biomarker of cardioembolic stroke it is necessary to know its short term kinetic after stroke in order to determine the best time to measure it. Currently, there is scarce and contradictory data about the best time to measure NT-proBNP after stroke [9,10].

In this study, we aimed to characterise NT-proBNP serum levels in the first three days after ischemic stroke.

Methods

Type of study

Observational, cross-sectional study

Study Population

We studied a consecutive sample of patients with ischemic stroke according to the World Health Organization criteria [11] or transient ischemic attack (TIA) [12] that were admitted to the Stroke Unit of the Neurology Department of the Hospital de Santa Maria, Lisboa, from December 2009 to June 2010 and from October 2010 to December 2010. In order to be included the patients had to be admitted within 24 hours of stroke onset and have blood collections done at 24, 48 and 72 hours after stroke onset. Patients were excluded if they had acute renal failure or chronic renal insufficiency (glomerular filtration rate glomerular determined by the equation of Cockcroft-Gault, less than 90 mL / min, hemodialysis or peritoneal dialysis).

Clinical Protocol

Patients with ischemic stroke were admitted from the Emergency Department and transferred to the Stroke Unit.

During hospital stay, information on demography, vascular risk factors, or previous atrial fibrillation was collected.

Hypertension was defined as a personal history of systolic blood pressure superior to 140 mmHg or/and diastolic blood pressure superior to 90 mmHg in two time periods before hospital admission or evidence of treatment with antihypertensive drugs.

The patient was considered to have diabetes mellitus whenever there was a previous diagnosis of diabetes mellitus, treatment with oral antidiabetics or insulin or there was after hospital admission a fasting blood glucose >126 mg/dL or/and post-prandial blood glucose > 200 mg/dL and/or tolerance test with blood glucose levels >200 md/dL at the 2º hour) or A1C > 6.5%.

Smoking was considered whenever there was a current or past history of smoking.

The patient was considered to have dyslipidemia if there was a previous diagnosis of hypercholesterolemia or hypertriglyceridemia or treatment with lipid-lowering drugs

The establishment of previous atrial fibrillation implied a personal history of atrial fibrillation or electrocardiographic evidence of this arrhythmia previous to the current hospital admission

Ischemic heart disease was defined as a personal history of angor, myocardial infarction or coronary revascularization.

Anemia was defined as hemoglobin levels below 12.0 g/dL.

Treatment at the time of stroke onset with drugs that could eventually cause changes in NT-proBNP serum levels such as angiotensin-converting enzyme inhibitors (ACEI), Angiotensin II receptor blockers (ARB), diuretics and Beta-blockers was also registered.

NT-proBNP measurements

In patients who complied with the study criteria, 4 ml of blood were drawn from a peripheral vein, at the first 24 (Day 1), 48 (Day 2) and 72 hours (Day 3) after stroke onset. Whenever

stroke symptoms were first noticed on awakening the time of stroke onset was considered as the time when the patients had last been seen asymptomatic. Blood samples were immediately taken to the Clinical Pathology Department to measure serum levels of NT-proBNP. NT-proBNP levels were measured by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) [13]. In this assay there is a simultaneous reaction between a polyclonal biotinylated NT-proBNP antibody and a ruthenium labeled polyclonal NT-proBNP antibody. These antibodies create a "sandwich" complex with the NT-proBNP that is present in the blood. The complex antigen-antibody is captured by a biotin-streptavidin reaction. The mixture that results from the reaction is aspired into a reading cell where the microparticles are magnetically captured to the surface of an electrode. The application of voltage to the electrode induces an emission that is measured. The software that is included in the system converts the intensity of the signal in a quantitative value.

A cut-off of NT-proBNP of 300 pg/ml has been suggested for suspicion of congestive heart failure [14]. The assay has a coefficient of variation in the range of 1.0 to 6.0%.

Stroke etiology determination

Laboratorial etiological workup included in all patients determination of a complete blood count, erythrocyte sedimentation rate, hepatic function, glucose and lipid levels, creatinine, protein electrophoresis, prothrombin time and activated partial thromboplastin time. In patients less than 55 years old, laboratorial workup also included evaluation of autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antithrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies.

Regarding neuroimaging, in all patients a brain-CT or Magnetic Resonance Imaging including diffusion-weighted imaging (MRI) (80.2%) was performed.

To study the intracranial and extracranial vessels all patients underwent transcranial Doppler and carotid and vertebral duplex scanning. Twenty eight patients (27,7%) had Magnetic Resonance Angiography. In 16 patients (15.8%) digital cerebral angiography was performed.

All patients underwent transthoracic echocardiogram. This exam was done blinded to NT-proBNP levels. The following parameters were registered: left atria dimensions, ejection fraction, presence of left atrial spontaneous echo contrast, intracardiac thrombus, patency of patent foramen oval (PFO), diastolic dysfunction [15] and hypokinetic/akinetic left ventricular segments. Systolic dysfunction was defined as a left ventricular ejection fraction <50%. In patients less than 55 years old a transesophageal echocardiogram was also performed. To diagnose atrial fibrillation, at least two ECGs and one 24-hours Holter monitoring were done during hospital stay.

Stroke etiological classification was done using the TOAST criteria [16]. Stroke subtypes were further grouped in cardioembolic and non-cardioembolic. The non-cardioembolic group included the subtypes of large-artery atherosclerosis, small vessels occlusion, stroke of other determined cause and stroke of undetermined cause.

The study was approved by the Ethic Committee of Hospital de Santa Maria, Lisbon, Portugal. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics

A descriptive statistical analysis of demographic and vascular risk factors of patients with ischemic stroke, cardioembolic and non-cardioembolic cause was performed. Data distribution was evaluated using histograms and a one sample Kolmogorov-Smirnov test. For normally distributed data, results were presented with mean and standard deviation (SD). For non-normally distributed data, median and interquartile ranges were defined. For comparison

between groups the chi-square test, Fisher exact test, Mann-Whitney test or T-test were used as appropriate.

To compare NT-proBNP values between the 3 different timings of blood collection, Friedman test was used. This was performed for all ischemic stroke patients and for those with cardioembolic and noncardioembolic stroke. Post-hoc analysis with Wilcoxon Signed-Rank Tests was conducted with a Bonferroni correction.

Mann-Whitney test was used to compare the median values of N-BNP between groups of patients with cardioembolic stroke vs noncardioembolic stroke.

Receiver Operating Curves and the Area Under the Curve to determine the accuracy of NT-proBNP to diagnose cardioembolic stroke were determined at each time point.

Statistical analyses were done using the *SPSS 19.0* for windows. Significance level was set at p=0.05.

Results

Between December 2009 – June 2010 and October 2010 – December 2010, 160 patients with ischemic stroke or TIA were admitted to the Stroke Unit. One hundred and one patients were included in the study (93 with stroke, 8 with TIA). Main reasons for patients' exclusion from the study were: admission more than 24 hours after stroke onset (n=26), unavailability of 3 measurements (n=14), less than 3 days of hospital stay (n=10), renal failure (n=1).

Included patients had a mean age of 64.5 years with a standard deviation (SD) of 12.3. Minimum age was 31 years and the maximum age was 86. Table 1 shows the characteristics of included patients. Classification of stroke etiology according to TOAST criteria is presented in Table 1. The main stroke etiology was undetermined followed by cardioembolic etiology. Other

determined etiology was due to artery dissection in two cases and hypercoagulable conditions in four cases (immunoglobulins infusion 1, iron deficiency anemia 1, antiphospholipid syndrome 1, and thrombocytosis 1).

Table 1 - Characteristics of the included patients

	All	Cardioembolic	Noncardioembolic	p
Patients (n)	101	29	72	
Age, years (mean +/- SD)	64.5 +/- 12.3	65.4 +/- 12.2	63.5 +/- 12.6	0.53
Gender, Female (n,%)	42 (41.6)	16 (55.2)	25 (34.7)	0.06
Hypertension (n,%)	71 (70.3)	19 (65.5)	52 (72.2)	0.51
Diabetes mellitus (%)	25 (24.8)	6 (21)	19 (26.4)	0.55
Dyslipidaemia (n,%)	33 (32.7)	8 (27.6)	25 (34.7)	0.49
Anemia (n,%)	11 (10.9)	6 (20.7)	5 (6.9)	0.07
ACEI or ARB (n,%)	50 (49.5)	13 (44.8)	37 (51.4)	0.55
Beta-blockers (n,%)	25 (24.8)	9 (31.0)	16 (22.2)	0.35
Diuretics (n,%)	31 (30.7)	8 (27.6)	23 (31.9)	0.67
Systolic dysfunction (n,%)	6 (6.0)	4 (13.8)	2 (2.8)	0.06
Diastolic dysfunction (n=76)	32 (42.1)	5 (23.8)	27 (49.1)	0.05
NT-proBNP Day 1 - Median - 1st quartile - 3rd quartile	 392.0 109.0 1077.5	 1203.0 827.0 2109.5	 170.5 66.5 510.8	 <0.001
NT-proBNP Day 2				

- Median	351	1607.0	177.5	<0.001
- 1st quartile	97	684.5	72.0	
- 3rd quartile	1132	2947.0	494.8	
NT-proBNP Day 3				
- Median	230	1380.0	144.0	<0.001
- 1st quartile	98	579.5	42.8	
- 3rd quartile	975	2390.0	288.8	
Etiology				
- Undetermined	34			
- Cardioembolic	29			
Atrial fibrillation	(18)			
Hypokinetic VS	(2)´			
Dil. Cardiomyop	(2)			
Atrial flutter	(1)			
Endocarditis	(2)			
PFO	(2)			
PFO/ASA	(1)			
Left atria smoke	(1)			
- Large vessels	28			
Intracranial	(12)			
Extracranial	(16)			
- Other determined	6			
- Small vessels	4			

SD – standard deviation, ACEI – angiotensin converting enzyme inhibitors, ARB - angiotensin receptor blockers, Hypokinetic VS – Hypokinetic ventricular segment, Dil. Cardiomyop – Dilated cardiomyopathy, PFO – Patent Foramen Ovale, ASA – Atrial Septal Aneurysm

Three hundred and three NT-proBNP determinations were done. The NT-proBNP serum determinations were done at exactly the following time points: mean (SD) 23h02m (1h51m), 46h50m (2h40m,) and 71h17m (2h42m). NT-proBNP values did not follow a normal distribution across the three time points (24, 48, 72 h). They had a positively skewed distribution. Median values of NT-proBNP were highest in the first 24 hours after ischemic stroke and decreased in the following time points The difference of NT-proBNP values across the 3 time points was statistically significant ($p<0.001$) (Figure 1). When we analyzed individual differences between the specific time points, there was no significant difference in NT-proBNP values at Day 1 versus Day 2 ($p=0.34$). Values of NT-proBNP were different at Day 2 versus Day 3 ($p<0.001$).

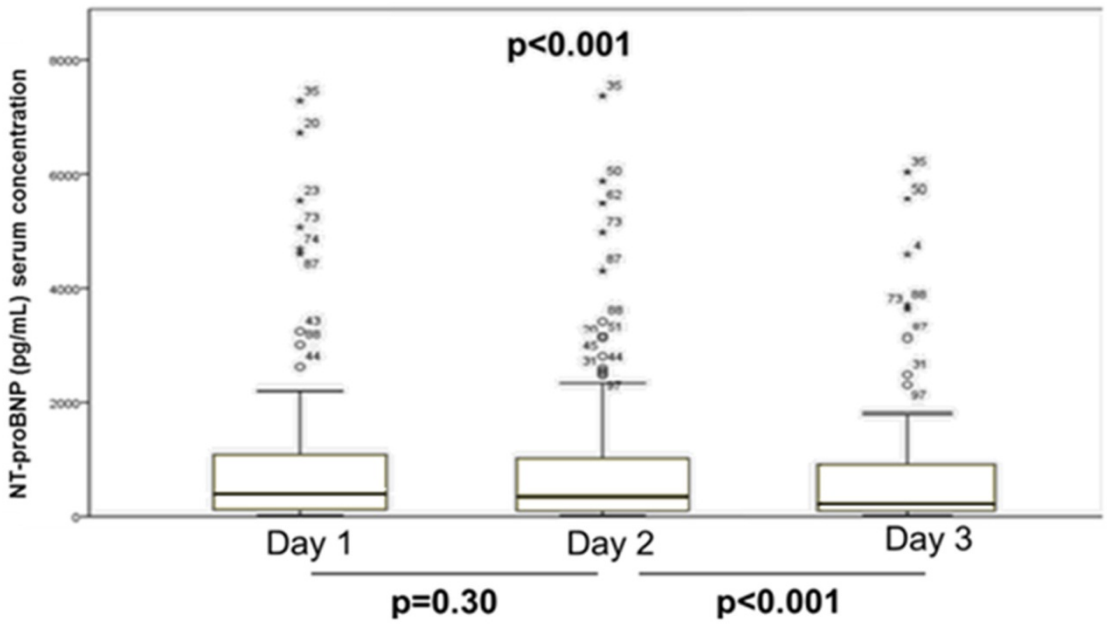


Figure 1 - NT-proBNP levels in the first 24, 48 and 72 hours in all patients. Boxplots present median values and interquartile ranges

NT-proBNP values in patients with cardioembolic stroke (n=29) were higher than in patients with non-cardioembolic stroke (n=72) in the three time points (p<0.001); (Table 1).

The same pattern of NT-proBNP values kinetic was observed in patients with cardioembolic and noncardioembolic stroke. NT-proBNP values were not significantly different in the first 2 days. However, in the third day there was a statically significant decrease, Cardioembolic Day 1 vs Day 2 vs Day 3 p=0.035, Day 1 vs Day 2 p=0.42, Day 2 vs Day 3 p=0.023, Noncardioembolic Day 1 vs Day 2 vs Day3 p<0.001, Day 1 vs Day 2 p=0.10, Day 2 vs Day 3 p<0.001 (Figure 2).

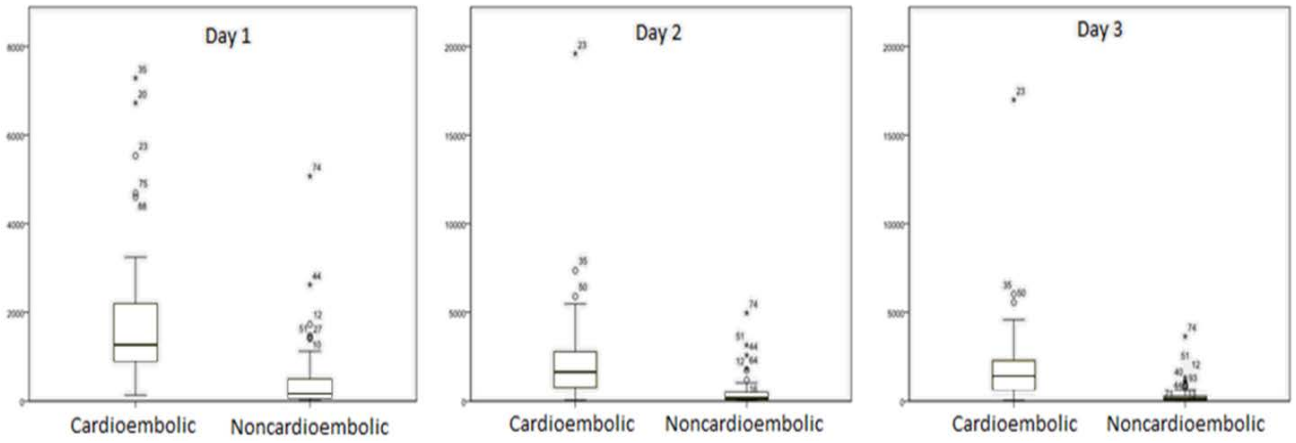
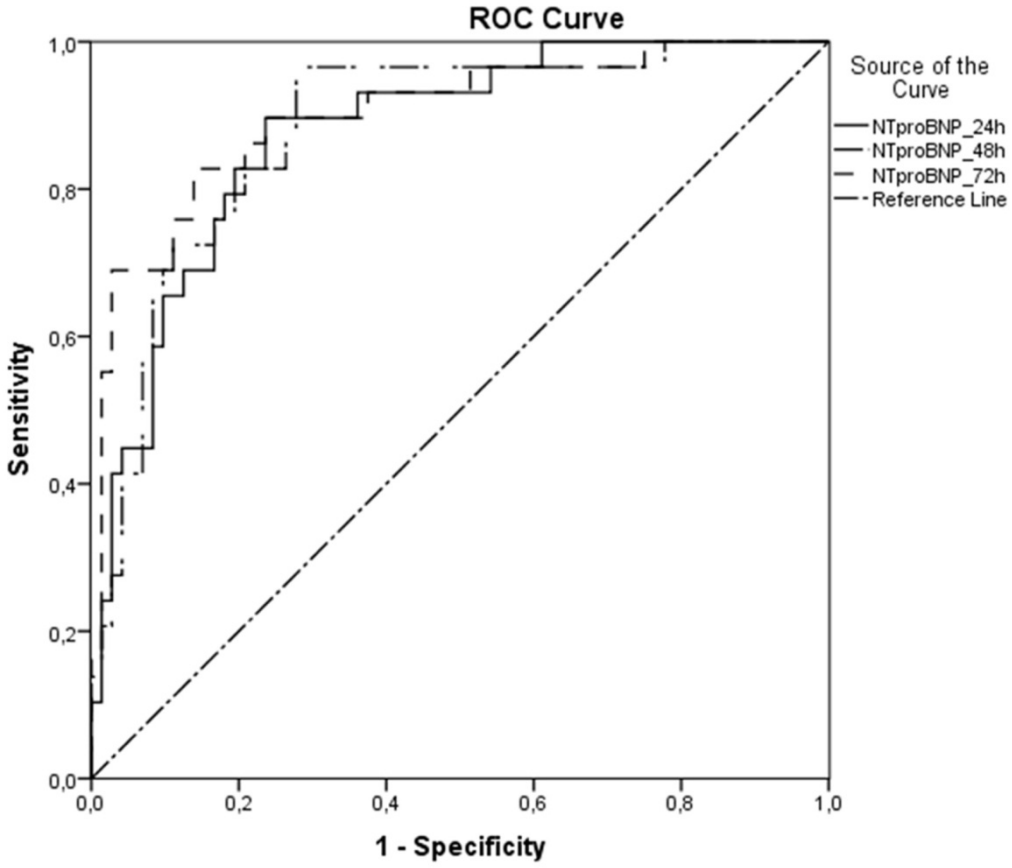


Figure 2 – Serum levels of NT-proBNP in patients with cardioembolic (n=72) and noncardioembolic stroke (n=29) in day 1-3. NT-proBNP values expressed in pg/mL

The Receiver Operating Curves of NT-proBNP at 24, 48 and 72 hours for the diagnosis of cardioembolic stroke had a similar very good Area Under the Curve (AUC) (Figure 3). The Areas Under the Curve values were for Day 1 - 0.88 (95% CI 0.81-0.95), Day 2 – 0.88 (95% CI 0.81-0.95), Day 3 – 0.90 (95% CI 0.83-0.97), p<0.001.

Despite the significant difference in the absolute values of the 3 times points, the Area Under the Curve of NT-proBNP at 72 hours was not inferior to the value at 24-48 hours.



	Area under the curve (c-statistic)	P-value	95% Confidence Interval
NT-proBNP 24h	0.876	<0.0001	0.806–0.947
NT-proBNP 48h	0.882	<0.0001	0.811-0.954
NT-proBNP 72h	0.902	<0.0001	0.832-0.972

Figure 3 – Receiver Operating Characteristic Curves and respective Areas Under the Curve (AUC) regarding the accuracy of NT-proBNP to diagnose cardioembolic stroke at 24, 48 and 72 hours after cardioembolic stroke. Areas Under the Curve values can vary between 0 and 1. A value of 0.5 means that the test is useless, 1 means that the test has a perfect diagnostic accuracy.

The number of patients with levels of NT-proBNP above the pre specified cut-off point with a high sensitivity for the diagnosis of cardioembolic stroke of 265.5 pg/mL was similar in the three time points (Figure 4)[5].

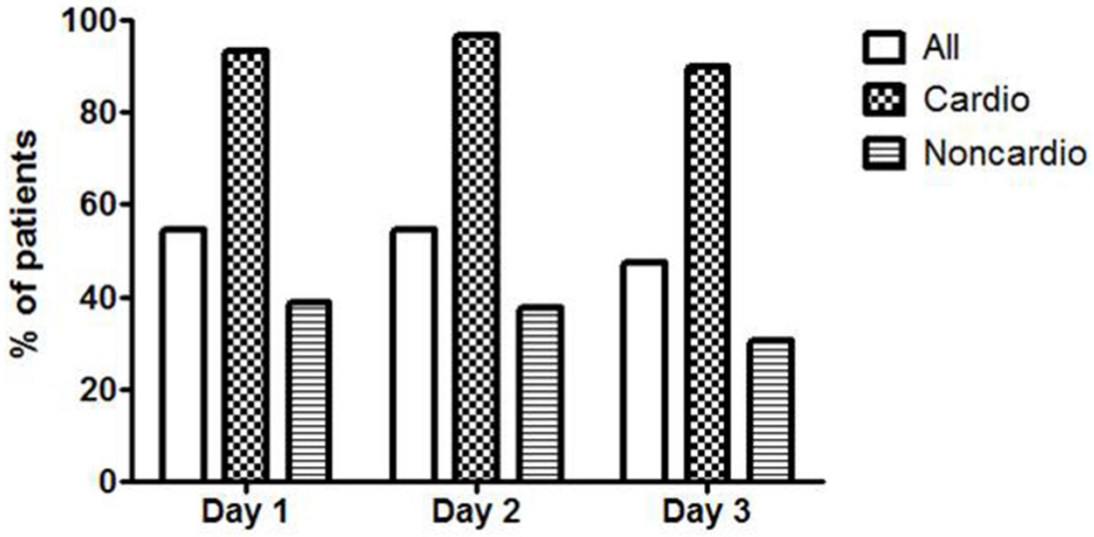


Figure 4 – Percentage of all patients, cardioembolic and noncardioembolic patients (including undetermined causes) with NT-proBNP values above the cut-off 265.5 pg/mL at day 1, day 2 and day 3.

Discussion

In all included stroke patients and in those with cardioembolic and noncardioembolic stroke (including undetermined causes), values of NT-proBNP were highest at 24 and 48 hours after ischemic stroke with no statistically significant difference between these two time points and had a statistically significant reduction 72h after stroke onset. However the area under the curve for the three time points showed similar diagnostic accuracy, which suggests that measurements of NT-proBNP in first 72 hours after ischemic stroke are equally useful.

Previously, two reports in literature analyzed the variation of NT-proBNP in the first days after ischemic stroke [9,10]. Itumur [10] measured NT-proBNP levels in 57 patients at day 1, 3, 5 and 10 after ischemic stroke. NT-proBNP levels were highest in the first day after ischemic stroke and declined significantly from day 3 onwards. However, there was no information about day 2 after stroke and individual stroke etiologies were not analyzed. Jensen [9] measured NT-proBNP levels in 250 patients at day 1, 2, 3, 4 and 5 after ischemic stroke. He found highest levels at day 2, with a significant difference between day 1 and day 2. Thereafter there was a progressive decreased until day 5. However, there was no information regarding the specific etiologies of stroke in these patients or the diagnostic accuracy of NT-proBNP in different time points. Patients with atrial fibrillation were excluded from the study.

In previous reports that studied NT-proBNP or BNP as a possible biomarker of cardioembolic stroke, measurements have been done during the first 24 hours after ischemic stroke [6,7,17]. The knowledge of an extended time window (72 hours) for the determination of NT-proBNP might be useful as a significant proportion of patients does not go to the hospital in the first 24 h after stroke onset [18]. This was a major cause of exclusion of patients in our cohort. The possibility of an increased time window for NT-proBNP measurements is an important possible advantage of the use of NT-proBNP instead of BNP in this setting, as NT-pro BNP is known to have an increased half-life [19]. This extended time window of 72 hours, can also have an advantage over the user of D-dimers for the diagnosis of cardioembolic stroke, which were found to be potentially useful for the diagnosis of cardioembolic stroke in the first 12 hours after ischemic stroke [20].

One limitation of our study was that only three time points with intervals of 24 hours were analyzed, eventually if shorter intervals were chosen they would be even more informative.

The proportion of undetermined stroke etiology in our cohort is similar to the proportion stated in other studies – a range from 30 to 40% [21,22]. Although 20 (19.8%) patients presented with a clinical lacunar syndrome in only 4 patients it was due to small vessels disease. This was due

to a high rate of use of DWI-MRI. DWI-MRI improves the accuracy of the subtype diagnosis of stroke [23]. Most cases of clinical lacunar syndromes not associated to small vessels disease were due to large artery or cardiogenic embolic stroke.

Our observation of higher levels of NT-proBNP in patients with cardioembolic stroke than in noncardioembolic stroke is in accordance with previous studies. The finding of a statistically significant difference in NT-proBNP across the 3 times points and the very good value of accuracy to diagnose cardioembolic stroke reinforces the idea that NT-proBNP may be used as a biomarker of cardioembolic stroke. Serum biomarkers may have an important role in the diagnosis of paroxysmal AF, even if ongoing trials of implantable event detectors have successful results [24]. To obtain NT-proBNP serum measurements it is necessary to perform a venipuncture but this is a less invasive procedure than the implantation of an event detector. NT-proBNP measurements are also less expensive and are widely available in most wards and emergency departments.

They could potentially be incorporated in a subclassification of patients with undetermined etiology and help in the stratification of patients that could benefit the most from long term heart rhythm monitoring.

Conclusion

NT-proBNP levels were highest 24-48 hours after ischemic stroke and started to decline thereafter. However the diagnostic accuracy of NT-proBNP to diagnose cardioembolic stroke was similar and very good in the first 72 hours after stroke onset. Our study reinforces that BNP may be useful in the identification of patients with cardioembolic stroke and in the etiological classification of TIA and stroke.

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2. Validation of NT-proBNP cut-off values for the diagnosis of cardioembolic stroke related to atrial fibrillation

Part of this chapter will be published in the following article:

- Fonseca AC, Brito D, Pinho e Melo T, Geraldes R, Canhão P, Caplan LR, Ferro JM. NT-proBNP shows diagnostic accuracy for detecting atrial fibrillation in cryptogenic stroke patients. Int J Stroke. *In press*

And was presented as an oral communication at the International Stroke Conference 2013

- Fonseca AC, Matias JS, Pinho e Melo T, Geraldes R, Canhão P, Brito D, Caplan LR, Ferro JM. Stroke 2013;44:A32.

Introduction

Stroke related to atrial fibrillation tends to be severe, with high recurrence and mortality rates [1]. Identification of atrial fibrillation in patients with stroke is consequently of paramount importance and has therapeutic implications. Atrial fibrillation is usually classified as permanent, persistent or paroxysmal. Paroxysmal atrial fibrillation (pAF) is reported to have the same risk as persistent or permanent atrial fibrillation to cause ischemic strokes [2].

However identifying pAF can be difficult. Although it is frequently related to structural heart disease, about 45% of patients with paroxysmal atrial fibrillation have no echocardiographic detectable heart disease [3]. The current available electrophysiological methods (ECG, routine telemetry during inpatients admission, Holter monitoring, 30-day event monitoring devices) to detect paroxysmal AF all have a low sensitivity [4]. If diagnostic tests fail to identify pAF, stroke etiology may be incorrectly classified as cryptogenic (undetermined etiology) [5].

Alternative ways to detect paroxysmal atrial fibrillation in cryptogenic stroke should be considered. Recent studies suggest that NT-proBNP (N-terminal probrain natriuretic peptide), a peptide produced by the heart, may be useful to identify cardioembolic stroke associated with AF [6,7,8]. In a previous study, NT-proBNP had good accuracy in predicting ischemic stroke of cardioembolic cause associated with atrial fibrillation. Two cut-off points – 265.5 pg/mL and 912.0 pg/ml, associated with a high negative and high positive predictive value for the diagnosis of AF (97.2% and 90.9 %, respectively) were obtained [6]

In this study we aimed to validate the previous defined cut-off values of serum NT-proBNP for the diagnosis of the presence of atrial fibrillation in patients with ischemic stroke.

Methods

Study Type:

Observational, cross-sectional study

Study Population:

Consecutive patients admitted to the Stroke Unit of the Neurology Department of the Hospital de Santa Maria from December 2009 to June 2010, from September 2010 to December 2010 and from April 2011 to March 2012. In order to be included patients had to have an ischemic stroke (according to the World Health Organization criteria [9]) or transient ischemic attack (TIA) [10] and be admitted to the stroke unit within 72 hours of stroke onset. Patients were excluded if they had acute renal failure or chronic renal insufficiency (glomerular filtration rate glomerular determined by the equation of Cockcroft-Gault, less than 90 mL / min, hemodialysis or peritoneal dialysis).

Sample size calculation:

To validate the cut-off values of NT-proBNP in patients with a defined stroke etiology, taking in account the previous evaluated sensitivity of 94.4% of the cut-off value of 265.5 pg/mL and the specificity of 97% of the cut-off value of 912.0 pg/mL [6], in a population where the prevalence of atrial fibrillation is 0.30, with a required lower 95% confidence limit >0.8 with 0.95 probability, a number of 50 patients and 116 controls was required (total 166 patients with a defined stroke etiology) [11].

Clinical Protocol:

Patients with ischemic stroke or transient ischemic attack (TIA) were admitted to the Stroke Unit. In patients who met the study criteria, 4 ml of blood were drawn from a peripheral vein within 72 hours of stroke onset. Blood samples were immediately taken to the Clinical Pathology Department. Blood samples were centrifuged at 1600 g during 15 minutes. NT-proBNP levels were measured by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) [12].

Information on demography, vascular risk factors, previous AF and admission National Institutes of Health Stroke Scale (NIHSS) score [13] was collected. Etiological workup included in all patients a complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis and coagulation studies. In patients <55 years old, workup also included autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antithrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies. In all patients a brain-CT or MRI (71% MRI), transcranial Doppler, carotid and vertebral duplex scanning and Transthoracic Echocardiographic examination (M-mode, two dimensional and Doppler study) were performed. The following parameters were registered: atrial dimensions (atrial dilatation was defined as an end-systolic diameter >40 mm) [14], left atrial spontaneous echo contrast, intracardiac thrombus, left ventricular ejection fraction (LVEF), persistence of patent foramen ovale (PFO), hypokinetic ventricular wall regions, diastolic dysfunction [15] and left ventricular hypertrophy. Systolic dysfunction was defined as a LVEF < 50%. In all patients <55 years old (18.7% of total patients), contrast transcranial Doppler with agitated saline was done to look for a right left shunt and transesophageal echocardiogram were performed. To detect pAF at least two ECGs and one 24-hours Holter monitoring were performed within the first week of the presenting event. Stroke etiological classification was done by stroke neurologists using the TOAST criteria, blind to NT-proBNP determinations [16]. If patients had a past history of atrial fibrillation, stroke was considered to

be cardioembolic if no other finding was disclosed. Atrial fibrillation was defined as a dysrhythmia with at least 30 continuous seconds [17] with no detectable P waves and no other diagnosis.

The study was approved by the Ethic Committee of our hospital. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics:

A descriptive statistical analysis of demographic and vascular risk factors of all patients with ischemic stroke, atrial fibrillation and non-atrial fibrillation was performed. Data distribution was evaluated using histograms and a one sample Kolmogorov-Smirnov test. For normally distributed data, results were presented with mean and standard deviation (SD). For non-normally distributed data, median and interquartile ranges (IQR) were defined. For comparison between groups the chi-square test, Fisher exact test, Mann-Whitney test or T-test were used as appropriate.

Patients with a defined stroke etiology at the time of hospital discharge were used to construct a Receiver Operating Characteristic (ROC) curve to determine the accuracy of NT-proBNP for the diagnosis of atrial fibrillation. In this case the gold standard for the diagnosis of atrial fibrillation was a known and established diagnosis based on a previous ECG or Holter. From this curve, the Area Under the Curve (c-statistics), the sensitivity and specificity - with the respective confidence intervals of the established cut-off points was calculated [6]. The cut-off points of NT-proBNP of 265.5 and 912.0 pg/mL were chosen in a previous study due to their high sensitivity (94.4%) and specificity (97.9%) respectively [6]. A multivariate analysis was performed using a logistic regression model, to assess the independent relationship of logNT-proBNP with atrial fibrillation controlling for previously known confounders – age, sex, atrial dilatation, left ventricular hypertrophy and systolic dysfunction. Variables were included in the logistic regression model as predictors based on prior knowledge and also based on the previous

study of the association between the different variables and outcome or exposure. Results are presented as odds ratios and 95% confidence intervals (CI).

Statistical analyses were done using the SPSS 19.0 and SAS 9.2 for windows. Significance level was set at p=0.05.

This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) statement procedures [18].

Results

264 patients were included with a mean age of 63.8 (SD 12.2) years (Figure 1).

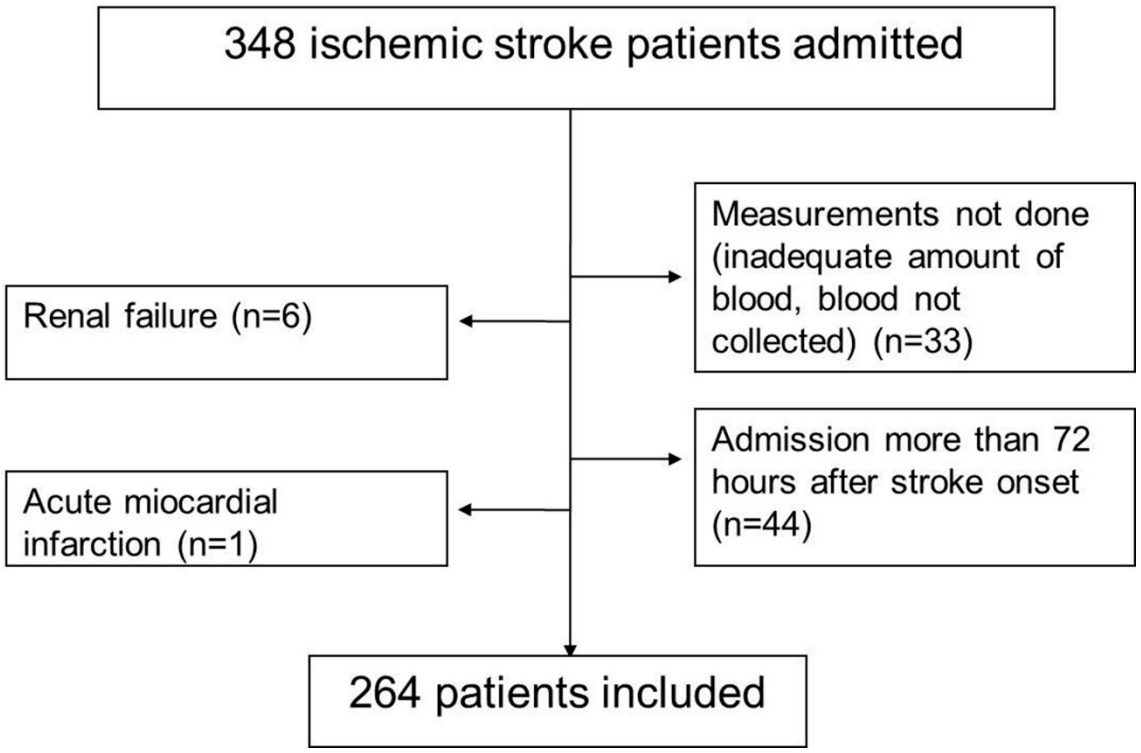

Figure 1 – Fluxogram of included patients

Twenty-five patients had a TIA (9.5%). In 184 patients a specific stroke etiology was established. The specific stroke etiologies were: 94 cardioembolic (51.1%), 57 large vessels disease (31.0%), 17 small vessels disease (9.2%), 6 undetermined/more than 1 possible etiology (3.3%). Among cardioembolic strokes 52 were due to atrial fibrillation, 4 due to a patent foramen ovale plus atrial septum aneurysm, 19 due to patent foramen ovale, 11 due to hypokinetic/akinetic left ventricular segments, 3 due to endocarditis, 1 due to mitral valve prolapse, 2 due to a mechanical valvular prosthesis, 1 due to dilated cardiomyopathy and 1 due to a heart tumor. Three of the 6 patients with more than a possible stroke etiology had both atrial fibrillation and an ipsilateral carotid stenosis superior to 50%. In total 55 patients had atrial fibrillation. Patients with atrial fibrillation were more frequently older, female, with atrial dilatation and higher admission NIHSS scores (Table 1). Patients without atrial fibrillation were more frequently smokers.

	AF (n=55)	No AF (n=129)	p-value
NT-proBNP (pg/dL) median (IQR)	1777 (2710)	138 (332)	<0.0001
TIA n (%)	2 (3.6)	18 (14.0)	0.04
Age, years mean (SD)	70.3 (7.8)	58.6 (12.7)	<0.0001
Female n (%)	31 (56.4)	48 (37.2)	0.02
Hypertension n (%)	36 (65.5)	91 (70.5)	0.49
Diabetes n (%)	10 (18.2)	37 (28.7)	0.14
Dyslipidemia n (%)	29 (52.7)	54 (41.9)	0.18
Current Smoker n (%)	4 (7.3)	39 (30.2)	0.001
Previous MI or angina n (%)	12 (21.8)	18 (14.0)	0.19
Previous stroke or TIA n (%)	21 (38.2)	30 (23.3)	0.04
Hgb (g/dL) mean (SD)	14.0 (1.7)	13.9 (1.8)	0.71
Weight (Kg) median (IQR)	74.1 (19.5)	75.0 (14.6)	0.22
ACEI n (%)	16 (29.1)	33 (25.6)	0.62

ARB n (%)	11 (20.0)	22 (17.1)	0.63
B-blocker n (%)	23 (41.8)	23 (17.8)	0.001
Admission NIHSS median (IQR)	13 (12)	4 (8)	<0.0001
Anterior circulation stroke n (%)	53 (96.4)	89 (70.6)	<0.0001
Atrial dilatation n (%)	42 (76.4)	31 (24.0)	<0.0001
LV hypertrophy n (%)	21 (38.2)	33 (25.6)	0.09
Systolic dysfunction n (%)	10 (18.2)	14 (10.9)	0.18
Diastolic dysfunction n (%) (n=115)	6 (21.4)	47 (54.0)	0.003
Segmental LV hypokinesia or akinesia n (%)	10 (18.2)	17 (13.2)	0.38

Table 1 – Characteristics of patients with a known stroke etiology, with or without atrial fibrillation; AF – atrial fibrillation, ACEI – angiotensin converting enzyme inhibitors, ARB - angiotensin receptor blockers, Hgb- hemoglobin, IQR – interquartile range, LV – left ventricular, MI – myocardial infarction, SD – standard deviation, TIA – transient ischemic attack; Systolic dysfunction was defined as a left ventricular ejection fraction < 50%,

NT-proBNP levels followed a right skewed distribution. Levels of NT-proBNP were higher in patients with atrial fibrillation than in patients without atrial fibrillation (Figure 2).

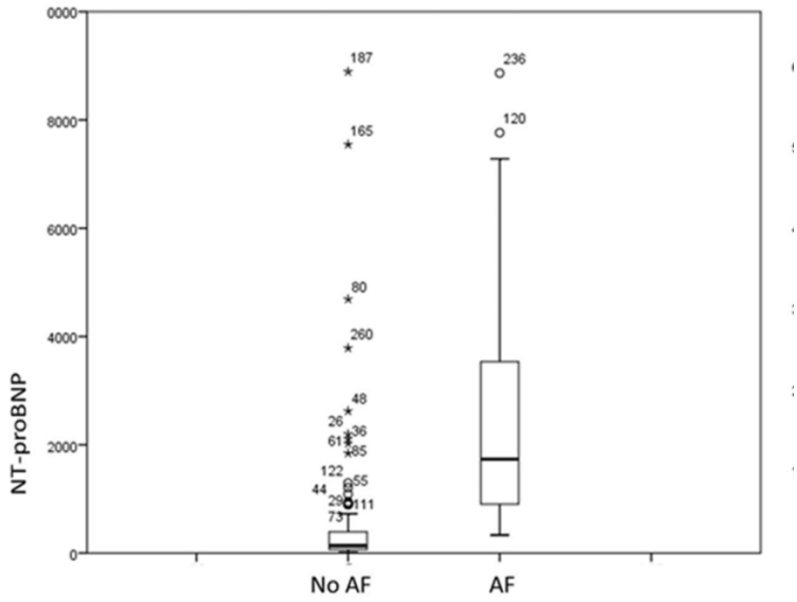

Figure 2 – NT-proBNP (pg/mL) levels in patients with a specific stroke etiology, with or without atrial fibrillation (AF)

Correlation of values of NT-proBNP levels with vascular risk factors and echocardiographic parameters are shown in table 2.

	NT-proBNP cut-off 265.5 pg/mL	
	Phi coefficient	p value
Sex	0.111	0.131
Hypertension	0.066	0.370
Diabetes Mellitus	0.031	0.674
Atrial fibrillation	0.646	<0.001
Atrial enlargement	0.529	<0.001
Left ventricular systolic dysfunction	0.319	<0.001
Left ventricular hypertrophy	0.232	0.003

Table 2 – Correlation of NT-proBNP values with vascular risk factors and echocardiographic parameters

Using multivariate analysis logNT-proBNP (logarithm transformation of NT-proBNP) was independently associated to atrial fibrillation (OR – 2.65, 95% CI 1.57-4.45; p<0.0001), after adjusting for age, sex, left ventricular hypertrophy, atrial dilatation and systolic dysfunction (Table 3).

	Atrial fibrillation		
	OR	95% CI	p-value
Log NT-proBNP	3.031	1.972-4.658	<0.0001
Female	4.949	2.047-11-96	<0.0001
Atrial dilatation	4.390	1.811-10.462	0.001
Age	1.054	1.008-1.103	0.021
Left ventricular hypertrophy	0.775	0.329-1.823	0.559
Systolic dysfunction	0.531	.0129-2.180	0.380

Table 3 - Results of the logistic regression

Adding admission NIHSS scores to the model did not change the significance of the association between logNT-proBNP and atrial fibrillation. Among patients with a specific stroke etiology, the Area Under the Curve (AUC) of the ROC curve of NT-proBNP for the diagnosis of atrial fibrillation was excellent - 0.91, 95% CI (0.87-0.95) (Figure 3). An AUC of 1 means that the test has a perfect diagnostic accuracy, 0.5 means that it is useless.

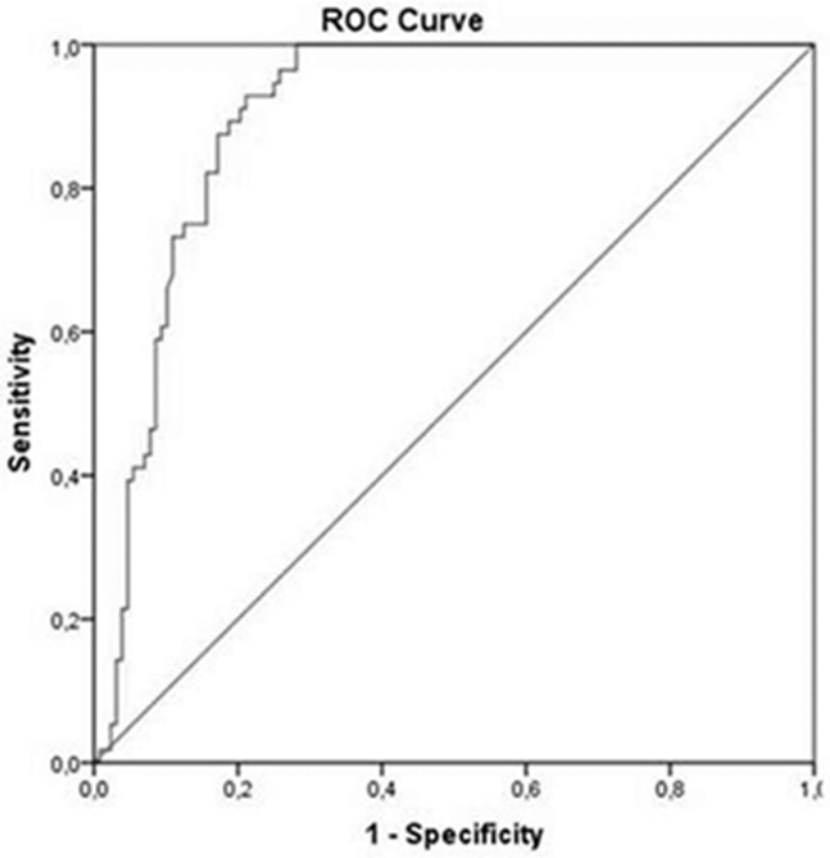

Figure 3 – ROC curve of NT-proBNP for the diagnosis of atrial fibrillation (AF) in patients with known stroke etiology

The previously defined cut-off of 265.5 pg/ml had a sensitivity of 100% 95% CI (93.5-100%), a specificity of 70.5% (62.2-77.7%), a positive predictive value of 59.1% 95% CI (48.5-69.2%) and a negative predictive value of 100% 95% CI (96.0-100%). In this study, the cut-off value of 304 pg/ml had the same sensitivity 100% 95%CI (93.5-100%) with a higher specificity of 71.3% 95% CI(62.7-78.9%), positive predictive value of 59.8% 95% CI (49.0-69.9%) and negative predictive value of 100% 95% CI (96-100%). The cut-off point of 912 pg/ml had a sensitivity of 81.8% 95% CI (69.7-89.8%), a specificity of 87.5% 95% CI (80.8-92.2%), a positive predictive value of 73.8% 95% CI (60.9-84.2%) and a negative predictive value of 91.9% 95% CI (85.6-96.0%). (Figure 4).

265.5 pg/mL

	AF	No AF
>	55	41
<	0	88

912.0 pg/mL

	AF	No AF
>	45	18
<	10	111

Figure 4 - Distribution of patients with a specific stroke etiology according to the defined cut-off point and the presence or not of atrial fibrillation (AF)

Discussion

This was the first study in which previously defined NT-proBNP cut-off levels for the diagnosis of cardioembolic stroke associated to atrial fibrillation were evaluated. In order to use NT-proBNP as a biomarker of cardioembolic stroke it is necessary to demonstrate the internal and external validity of the cut-off points. The initial study tends to optimize the defined cut-off points, therefore the sensitivity and specificity of the cut-off points tends to fall in validation studies. In order to validate the cut-off points a sample size was calculated. When the cut-off points were evaluated in this second study their high sensitivity and specificity was maintained. This is a point in favor of their external validity, however the cut-off points should be further validated in studies involving patients from different centers and with different baseline characteristics, to confirm if these results apply to other patient groups. The normal range of

NT-proBNP varies on the basis of age and gender, making it difficult to define a true reference range. Nevertheless, normal ranges of NT-proBNP fall well below our defined cut-off points levels of NT-proBNP level for patients with atrial fibrillation.

As the question if NT-proBNP was an independent predictor of atrial fibrillation or if the increase of NT-proBNP was just do to concomitant systolic or diastolic dysfunction aroused it was necessary to perform a multivariate analysis. The variables used in the logistic regression model included factors known to be possible confounders, this is related to both NT-proBNP increase and to atrial fibrillation. After controlling for age, sex, atrial dilatation, left ventricular hypertrophy and systolic dysfunction, NT-proBNP was independently associated to atrial fibrillation. Hypertension and diastolic dysfunction were not put into the model because they are highly correlated to the other variables (left ventricular hypertrophy and systolic dysfunction). The result that NT-proBNP is an independent predictor of atrial fibrillation after controlling for systolic dysfunction and atrial enlargement is in agreement with previous studies. One study demonstrates that NT-proBNP level was directly and proportionately associated with AF burden as assessed by continuous ambulatory rhythm monitoring and that this association was independent of other factors known to affect NT-proBNP. There are recent publications that suggest that NT-proBNP is a predictor of atrial fibrillation following cardiac surgery [21,22,23] and successful cardioversion [24]. The results support the notion that atrial fibrillation is an additional, independent cause of elevated NT-proBNP levels and should be part of the differential diagnosis when increased levels are present.

The logistic regression model shows that several of the included variables were independently related to atrial fibrillation. From the results of the logistic regression a predictive score containing clinical, laboratorial and echocardiographic characteristics could be derived, nevertheless this was not an aim of this specific study. We intended to determine a cut-off point that could increase the physician suspicion of atrial fibrillation and guide the execution of further electrophysiological tests to confirm the diagnosis of paroxysmal atrial fibrillation. The

results suggest that knowing the result of NT-proBNP serum levels could be useful in this setting.

Conclusion

NT-proBNP may be a useful surrogate for atrial fibrillation in ischemic stroke.

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3. NT-proBNP accuracy for detecting atrial fibrillation in cryptogenic stroke

Part of this chapter will be published in the following article:

- Fonseca AC, Brito D, Pinho e Melo T, Geraldes R, Canhão P, Caplan LR, Ferro JM. NT-proBNP shows diagnostic accuracy for detecting atrial fibrillation in cryptogenic stroke patients. Int J Stroke. *In press*

And was presented as an oral communication at the "7º Congresso Português do AVC"

- Fonseca AC, Matias JS, Pinho e Melo T, Geraldes R, Canhão P, Brito D, Ferro JM. Sinapse 2013;13(1):147.

Introduction

Alternative ways to detect paroxysmal atrial fibrillation in cryptogenic stroke should be considered. Recent studies suggest that NT-proBNP (N-terminal probrain natriuretic peptide), a peptide produced by the heart, may be useful to identify cardioembolic stroke associated with AF [1,2,3].

Elevated NT-proBNP is also a marker for patients at high risk of atrial fibrillation. In the Cardiovascular Health Study, a large cohort study of elderly people followed for cardiovascular outcomes, the risk of developing incident atrial fibrillation was increased in those with elevated NT-proBNP, but not during the initial 2 years [4].

In a previous study, NT-proBNP had good accuracy in predicting ischemic stroke of cardioembolic cause associated with atrial fibrillation. Two cut-off points – 265.5 pg/mL and 912.0 pg/ml, associated with a high negative and high positive predictive value for the diagnosis of AF (97.2% and 90.9 %, respectively) were obtained [1]

In this study we aimed to determine if NT-proBNP could identify paroxysmal AF in patients with strokes initially classified as cryptogenic.

Methods

Study Type:

Observational, prospective cohort study

Study Population:

Consecutive patients admitted to the Stroke Unit of the Neurology Department of the Hospital de Santa Maria, Lisboa, from December 2009 to June 2010, from September 2010 to December 2010 and from April 2011 to March 2012. In order to be included patients had to have an ischemic stroke (according to the World Health Organization criteria [5]) or transient ischemic attack (TIA) [6] and be admitted to the stroke unit within 72 hours of stroke onset. Patients were excluded if they had acute renal failure or chronic renal insufficiency (glomerular filtration rate glomerular determined by the equation of Cockcroft-Gault, less than 90 mL / min, hemodialysis or peritoneal dialysis).

Sample size calculation:

Taking into account the information from a previous publication [1] the mean of NT-proBNP levels in patients with an cardioembolic ischemic stroke was 491.6 pg/mL with a standard deviation of 420.96 (n=28) and the mean in patients with non-cardioembolic stroke was 124.7 pg/mL with a standard deviation of 69.55 (n=38). With a power of 95% and an alpha of 0.05 to show a statistically significant difference between groups, a total sample size of 40 patients with undetermined stroke etiology. Assuming a percentage of cryptogenic strokes of 16%, a cohort of 250 patients was needed.

Clinical Protocol:

Patients with ischemic stroke or transient ischemic attack (TIA) were admitted to the Stroke Unit. In patients who met the study criteria, 4 ml of blood were drawn from a peripheral vein within 72 hours of stroke onset. Blood samples were immediately taken to the Clinical Pathology Department. Blood samples were centrifuged at 1600 g during 15 minutes. NT-

proBNP levels were measured by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) [7].

Information on demography, vascular risk factors, previous AF and admission National Institutes of Health Stroke Scale (NIHSS) score [8] was collected. Etiological workup included in all patients a complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis and coagulation studies. In patients <55 years old, workup also included autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antithrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies. In all patients a brain-CT or MRI (71% MRI), transcranial Doppler, carotid and vertebral duplex scanning and Transthoracic Echocardiographic examination (M-mode, two dimensional and Doppler study) were performed. The following parameters were registered: atrial dimensions (atrial dilatation was defined as an end-systolic diameter >40 mm) [9], left atrial spontaneous echo contrast, intracardiac thrombus, left ventricular ejection fraction (LVEF), persistence of patent foramen ovale (PFO), hypokinetic ventricular wall regions, diastolic dysfunction [10] and left ventricular hypertrophy. Systolic dysfunction was defined as a LVEF < 50%. In all patients <55 years old (18.7% of total patients), contrast transcranial Doppler with agitated saline was done to look for a right left shunt and transesophageal echocardiogram were performed. To detect pAF at least two ECGs and one 24-hours Holter monitoring were performed within the first week of the presenting event. Stroke etiological classification was done by stroke neurologists using the TOAST criteria, blind to NT-proBNP determinations [11]. If patients had a past history of atrial fibrillation, stroke was considered to be cardioembolic if no other finding was disclosed. Patients with a cryptogenic stroke were followed as outpatients. During follow-up, in all patients, after hospital discharge, independently of NT-proBNP values, serial 24 hour Holter monitoring was performed within three and six months (if the previous monitoring was unremarkable) to look for paroxysmal atrial fibrillation and was used as reference standard. Atrial fibrillation was defined as a dysrhythmia with at least 30 continuous seconds [12] with no detectable P waves and no other

diagnosis. The study was approved by the Ethic Committee of our hospital. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics

A descriptive statistical analysis of demographic and vascular risk factors of all patients with ischemic stroke, atrial fibrillation and non-atrial fibrillation was performed. Data distribution was evaluated using histograms and a one sample Kolmogorov-Smirnov test. For normally distributed data, results were presented with mean and standard deviation (SD). For non-normally distributed data, median and interquartile ranges (IQR) were defined. For comparison between groups the chi-square test, Fisher exact test, Mann-Whitney test or T-test were used as appropriate.

To evaluate the diagnostic accuracy of NT-proBNP to diagnose paroxysmal atrial fibrillation in patients with cryptogenic stroke, Receiver Operating Characteristic curves were determined. The area under the curve (c-statistics) was determined.
Statistical analyses were done using the SPSS 19.0 and SAS 9.2 for windows. Significance level was set at p=0.05. This study followed the STARD statement procedures [13].

Results

The characteristics of the 264 patients that were included in this cohort were presented in the previous chapter. Among the 264 patients, 80 patients had a cryptogenic stroke at the time of hospital discharge. Three patients died within the first month of follow-up. These patients had NT-proBNP values of 1066, 1259 and 2426 pg/mL. In 17 (21.3%) paroxysmal atrial fibrillation was found during follow-up. In 14 patients the identification of paroxysmal atrial fibrillation was done within the first month after stroke onset. Patients in whom paroxysmal atrial fibrillation was subsequently found were older and had higher admission NIHSS scores (Table 1).

	AF (n=17)	No AF (n=63)	p-value
NT-proBNP (pg/dL) median (IQR)	883 (817)	214 (367)	<0.0001
TIA n (%)	0 (0)	5 (7.9)	0.58
Age, years median (IQR)	74 (7)	68 (15)	0.006
Female n (%)	13 (76.5)	25 (39.7)	0.12
Hypertension n (%)	12 (70.6)	45 (71.4)	0.99
Diabetes n (%)	4 (23.5)	19 (30.2)	0.77
Dyslipidemia n (%)	8 (47.1)	18 (28.6)	0.16
Current Smoker n (%)	0 (0)	7 (11.1)	0.34
Previous MI or angina n (%)	2 (11.8)	7 (11.1)	0.99
Previous stroke or TIA n (%)	3 (17.6)	15 (23.8)	0.75
Hgb (g/dL) mean (SD)	13.4 (1.0)	13.7 (1.4)	0.42
Weight (Kg) median (IQR)	70 (11.9)	74 (12.5)	0.22
ACEI n (%)	6 (35.3)	11 (17.5)	0.18
ARB n (%)	4 (23.5)	15 (23.8)	0.99
B-blocker n (%)	7 (41.2)	12 (19.0)	0.10
Admission NIHSS median (IQR)	13 (11)	7 (8)	0.04
Anterior circulation stroke n (%)	16 (94.1)	48 (76.2)	0.17
Atrial dilatation n (%)	10 (58.8)	21 (33.3)	0.06
LV hypertrophy n (%)	4 (23.5)	24 (38.1)	0.26
Systolic dysfunction n (%)	1 (5.9)	0 (0)	0.21
Diastolic dysfunction n (%) (n=60)	7 (53.8)	27 (57.4)	0.82

Table 1 – Characteristics of patients with cryptogenic stroke at the time of hospital discharge; AF – atrial fibrillation, ACEI – angiotensin converting enzyme inhibitors, ARB - angiotensin receptor blockers, Hgb- hemoglobin, IQR – interquartile range, LV – left ventricular, MI –

myocardial infarction, SD – standard deviation, TIA – transient ischemic attack; Systolic dysfunction was defined as a left ventricular ejection fraction < 50%

NT-proBNP values at admission were higher in cryptogenic stroke patients in whom paroxysmal atrial fibrillation was later found than in patients without detection of atrial fibrillation (Figure 1).

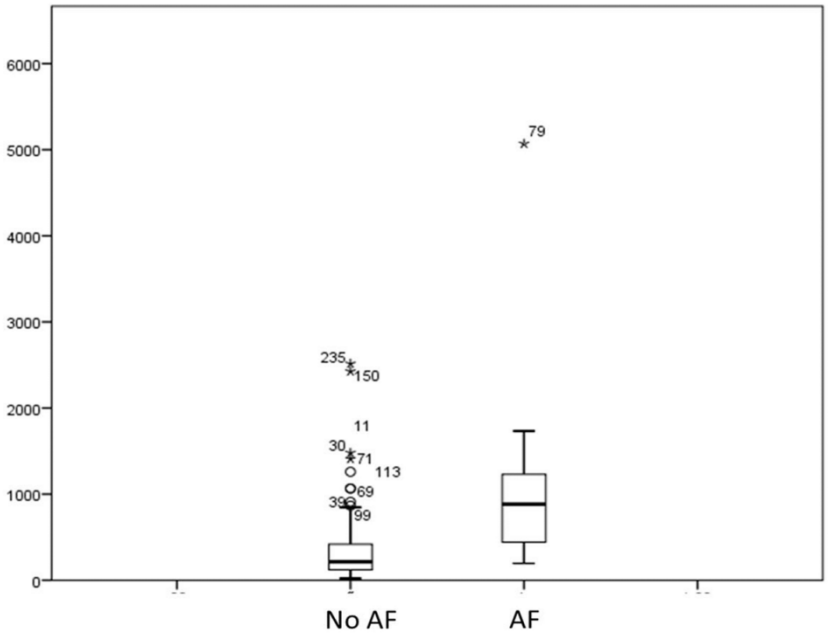

Figure 1 – NT-proBNP (pg/mL) levels in patients with initial cryptogenic stroke with or without later detection of paroxysmal atrial fibrillation (pAF)

In patients with cryptogenic stroke, the cut-off point of 265.5 pg/ml had a sensitivity of 88.2% 95% CI (65.7-96.7%), a specificity of 61.9% 95% CI (49.6-72.9%), a positive predictive value of 38.5% 95% CI (23.4-55.4%) and a negative predictive value of 95.1% (83.4%-99.3%) for the diagnosis of paroxysmal atrial fibrillation. The cut-off point of 912 pg/mL had a sensitivity of 47.1% 95% CI (26.2-69.0%) and a specificity of 88.9% 95% CI (78.8-94.5%), a positive

predictive value of 53.3% 95%CI (26.7-78.7%) and a negative predictive value of 86.2% 95%CI (75.3-93.5%) (Figure 2).

Cryptogenic stroke

265.5 pg/mL

	AF	No AF
>	15	24
<	2	39

912.0 pg/mL

	AF	No AF
>	8	7
<	9	56

Figure 2 – Distribution of patients according to the defined cut-off point and the presence or not of atrial fibrillation (AF)

The AUC of the ROC curve of NT-proBNP for the diagnosis of paroxysmal atrial fibrillation in patients with cryptogenic stroke was good - 0.83, 95% CI (0.73-0.92) (Figure 3).

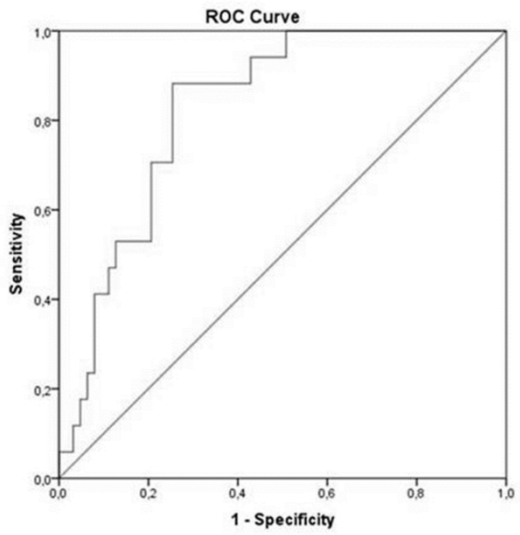

Figure 3 – ROC curve of NT-proBNP for the diagnosis of paroxysmal atrial fibrillation in patients with an initial cryptogenic stroke

Discussion

NT-proBNP levels at the time of stroke onset were helpful to identify paroxysmal atrial fibrillation in the follow-up of patients with an undetermined stroke etiology. The previously defined cut-off points of 265.5pg/mL and 912.0 pg/mL had a high sensitivity and specificity for the diagnosis of paroxysmal AF in patients with cryptogenic stroke (88.2 and 88.9% respectively). This is the first study in which NT-proBNP accuracy to diagnose paroxysmal atrial fibrillation was analyzed in cryptogenic stroke.

Normally atrial and ventricle myocytes express BNP genes that code for ProBNP. ProBNP is stored in atrial granules. Following the hemodynamic effect of atrial fibrillation, the precursor molecules, pre-produced proBNP, that are stored in atrial myocites are secreted as BNP and NT-proBNP [14], leading to the high levels of NT-proBNP. Therefore NT-proBNP levels could remain elevated in serum during sinus rhythm after a short course of paroxysmal atrial fibrillation. Probably patients with cryptogenic stroke in which later atrial fibrillation were

found had an episode of atrial fibrillation in close vicinity to stroke onset that caused an increase in NT-proBNP levels. Although they returned to a sinus rhythm, NT-proBNP remained elevated as a consequence of the paroxysmal episode of atrial fibrillation. NT-proBNP has been shown to be a remarkable predictor of incident atrial fibrillation in the general population, independently of any other previously described risk factor [15].

Although in this study median levels of NT-proBNP in patients with paroxysmal atrial fibrillation were lower than the median levels of NT-proBNP obtained in patients with persistent or permanent atrial fibrillation in the initially exploratory study [1] , the cut-off points still maintained a high sensitivity and specificity. A previous study also found higher levels of NT-proBNP in patients with persistent/permanent atrial fibrillation as compared with paroxysmal atrial fibrillation [4].

One report [16] evaluated NT-proBNP as a predictor of atrial fibrillation in patients with stroke in sinus rhythm in general and found that it had a reasonable accuracy for this diagnosis (AUC – 0.638 (95%CI 0.531-0.744)). Differences between this study and ours may explain the dissimilar result:

1) In our study we evaluated the diagnostic accuracy of NT-proBNP to diagnose atrial fibrillation in patients with cryptogenic stroke, while the study reported by Wachter et al [17] evaluated NT-proBNP accuracy to diagnose atrial fibrillation in stroke patients with sinus rhythm. The most clinically relevant question is which patients with cryptogenic stroke have atrial fibrillation. In the study of Wachter et al, patients with an already established cardioembolic etiology were included in the group of patients further evaluated for atrial fibrillation [17]. The cardioembolic etiologies of these patients were not included in the report [17]. These already known cardioembolic etiologies may have contributed to increased NT-proBNP levels in these patients.

2) We used different methods and a longer time periods to look for atrial fibrillation which might have influenced the results and lead to a higher rate of detection of atrial fibrillation (12.7 versus 21.3% in our study).

3) In our study we preferred to use NT-proBNP instead of BNP because NT-proBNP is known to have an increased half-life [14] and therefore may extend the time window during which it can be determined after stroke onset. This is clinically important because a sizable fraction of patients do not attend the emergency department in the first 24 hours after stroke onset

Strengths of this study include the following:

1) It is the first study that evaluated the accuracy of NT-proBNP to diagnose atrial fibrillation in patients with cryptogenic stroke. For this a follow up time of six months was used;

2) It was designed to take place in a clinical setting similar to the one in which it could be applied;

3) This study showed that the cut-off values for NT-proBNP that were defined previously were corroborated as being very sensitive and specific for paroxysmal atrial fibrillation;

4) The STARD criteria were followed.

Limitations of this study include the time points during the follow up after ischemic stroke that were chosen to look for atrial fibrillation. It is possible that in some patients with paroxysmal atrial fibrillation this arrhythmia was not diagnosed with the currently used diagnostic examinations. However a low detection rate would only contribute to decrease the sensitivity and specificity of the chosen points. Therefore, specificity might have been underestimated in this study. In patients with cryptogenic stroke the diagnosis of atrial fibrillation is particularly important as it changes the therapeutic decision, leading to the prescription of anticoagulants instead of antiplatelet agents. Cryptogenic stroke patients are many times submitted to extensive and costly electrophysiological investigations. NT-proBNP measurements could be helpful to

guide or eliminate further ECG, Holter or other electrophysiologic monitoring in patients with stroke. In patients with cryptogenic strokes, with uneventful echocardiograms an increase in NT-proBNP may point to the presence of paroxysmal atrial fibrillation and lead to further electrophysiological investigation.

Conclusion

NT-proBNP had good accuracy to predict paroxysmal atrial fibrillation in patients with cryptogenic stroke and might help in the evaluation of these patients.

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4. Potential of brain natriuretic peptide to identify TIA and stroke due to occult atrial fibrillation: population-based study

Part of this chapter was presented at the 20th European Stroke Conference, 2011

- Fonseca AC, Segal H, Burgess AI, Poole D, Rothwell PM. Potential of brain natriuretic peptide (BNP) to identify TIA and stroke due to occult atrial fibrillation: population-based study. Cerebrovasc Dis 2011;31(suppl 2):295.

Introduction

Natriuretic peptides such as BNP and NT-proBNP may be useful biomarkers for occult paroxysmal atrial fibrillation in patients with ischemic stroke of undetermined etiology. Using a population based study, we related baseline BNP levels at the time of a first transient ischemic attack or ischemic stroke to etiology in patients who had a recurrent ischemic stroke during follow-up.

We aimed to determine if patients with an undetermined ischemic stroke etiology in which latter on atrial fibrillation was found had high serum level of BNP at the time of the index stroke.

Methods

Type of study

Case control-study

Sample

Patients included in the Oxford Vascular Study (OXVASC) from 2002 to 2009 due to an initial ischemic stroke with a late recurrence (more than 90 days after the initial stroke). [1] The OXVASC study population consists of 91 106 individuals, registered with 63 family physicians in nine general medical practices in Oxfordshire, United Kingdom. In the United Kingdom, general practices provide primary health care for registered individuals and hold a lifelong record of all medical consultations (from the National Health Service and private health care), and details of treatments, blood pressure, and investigations. All participating practices held

accurate patient registers, and allowed regular searches of their computerized diagnostic coding systems.

In order to be included patients had to have:

- Evidence of an initial ischemic stroke according to the World Health Organization definition [2] and confirmation with a Head-CT or Brain-MRI
- Blood analysis at the time of the index stroke
- ECG at the time of index stroke
- Performance of at least one extra cranial vascular imaging
- At least one late ischemic recurrence (> 90 days)

Patients were excluded from the study if they had:

- Heart failure at the time of index stroke (modified Framingham criteria for heart failure (e) or injection fraction below 50% in a transthoracic echocardiogram)
- Chronic renal failure at the time of index stroke (glomerular filtration rate determined by the Cockcroft-Gault equation below 90 mL/min, hemodialysis or peritoneal dialysis)

Demographic variables, patients' personal history of hypertension, diabetes mellitus, atrial fibrillation, anemia, blood pressure lowering medication were recorded. Ischemic stroke etiology classification was done according to the TOAST classification.

Study design

We analyzed all patients in the OXVASC (2002-2009) with an transient ischemic attack or ischemic stroke who had a recurrent stroke more than 90-days after the initial event.

Stroke etiology was classified according to the data pertaining to the initial event using the TOAST criteria. Recurrences classified as undetermined etiology by TOAST were also classified (blind to BNP results) as probably cardioembolic versus probably non-cardioembolic

based on clinical and imaging criteria. Stroke were further classified has probably cardioembolic when there were large territorial infarcts, with a complete investigation. The definition of undetermined stroke was redefined for this study. Patients were considered as having had an undetermined stroke etiology when they had a normal complete investigation. Complete investigation was defined to be composed by blood analysis, ECG, Head CT or Brain MRI and at least one extracranial vascular imaging. Patients without a complete investigation were excluded. Patient with more than one possible etiology were classified in a separate group. Patients were treated at the time of stroke onset, concerning stroke secondary prevention, with the best medical therapy available at the time. Blood was drawn at the time of the first event and stored. The stored blood was used to determine BNP levels through an ELISA method.

Statistical analysis

A descriptive statistical analysis of demographic and vascular risk factors of all patients with ischemic stroke was performed. Data distribution was evaluated using histograms and a one sample Kolmogorov-Smirnov test. For normally distributed data, results were presented with mean and standard deviation (SD). For non-normally distributed data, median and interquartile ranges (IQR) were defined. For comparison between groups the chi-square test, Fisher exact test, Mann-Whitney test or T-test were used as appropriate.

Results

Ninety-nine patients had a recurrent stroke during follow-up. Their mean age was 77.3 years, with a minimum of 50 years, and a maximum of 80 years. Forty-seven were men. Median time to first recurrence was 1.45 years with a minimum of 0.29 years and a maximum of 6.8 years. Mean time of blood storage was 5.8 years with a standard deviation of 1.8 years.

Table 1 shows the etiological classification of the first and recurrent event.

		Stroke etiology of the late recurrent events								
		CE (AF)	CE (MI)	CE (hyp)	CE (HF)	LA	SMV	UND	SEVERAL	other
Stroke etiology of the first event	CE (AF)	28							1	1
	CE (MI)							1		
	CE (hyp)			1						
	CE (HF)									
	LA	2	1			2			1	
	SMV	1				1	3	3		
	UND	10		1		3	6	30	1	
	SEVERAL	1								
	other									1

Table 1 – TOAST classification of first and late events, AF – atrial fibrillation, CE – cardioembolic, HF – heart failure, Hyp – hypokinesia, LA – large vessels disease, MI – myocardial infarction, SMV – small vessels disease, UND – undetermined

Patients with known atrial fibrillation at the initial stroke (n=32) had a higher ($p<0.001$) median (IQR) BNP (1988; 788-3542 pg/mL) than those without atrial fibrillation (248; 97-1259 pg/mL).

Among patients with initially undetermined stroke etiology, those in whom atrial fibrillation was discovered at the time of the recurrent stroke (n=19) had had higher ($p=0.05$) baseline BNP (584;294-1467 pg/mL) than those (n=10) with a recurrent stroke of other definite etiology (173;95.4-337) (Figure 1).

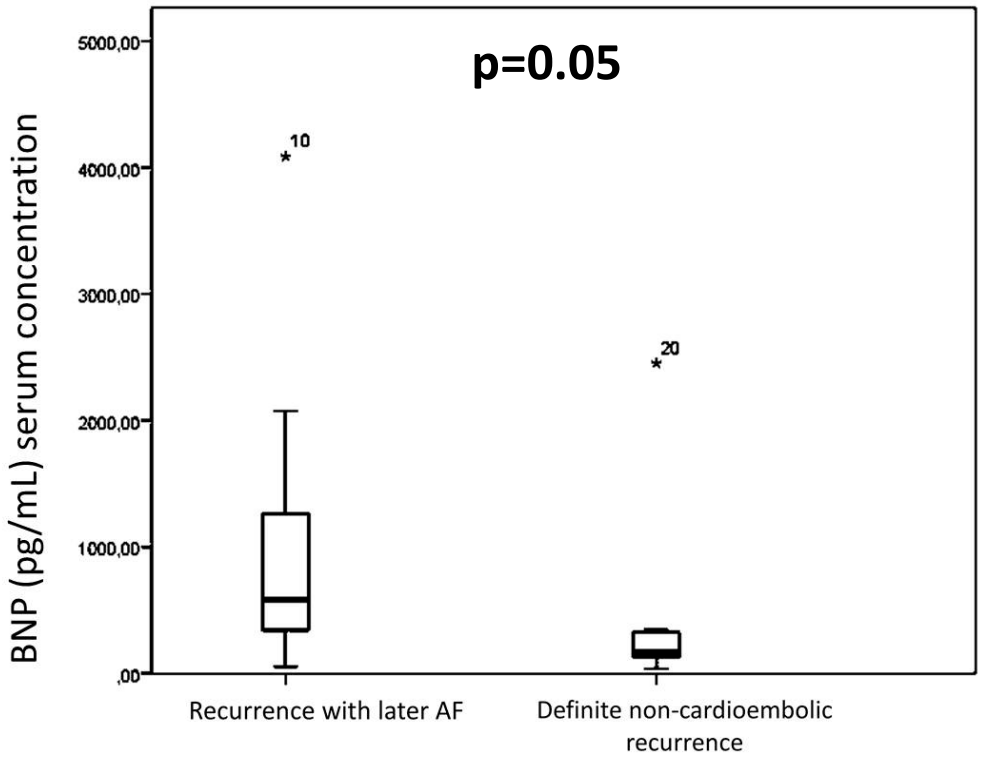

Figure 1 - Serum concentration of BNP at the initial event in patients that initially had an undetermined etiology and latter had a recurrence with atrial fibrillation versus patients that had a definite non-cardioembolic recurrence

The same was true in patients with initially undetermined stroke etiology with later atrial fibrillation or probable cardioembolic recurrence (n=27) versus those (n=24) with probable or definite non-cardioembolic etiology: 437 (154-1410) vs 159 (64-380); p=0.026 (Figure 2).

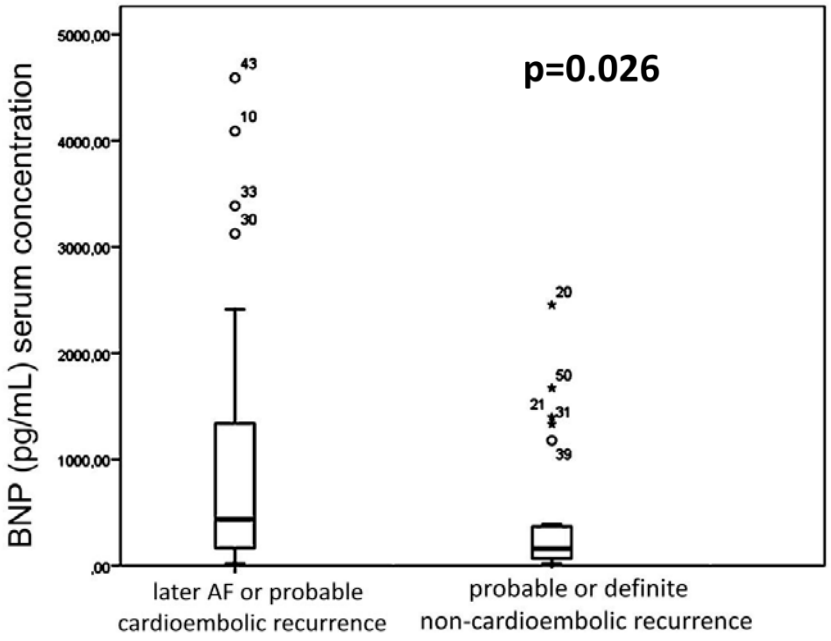

Figure 2 – Serum concentration of the BNP at the initial event in patients that initially had an undetermined etiology and latter had recurrence with AF or probable cardioembolic versus a probable or definite non-cardioembolic recurrence

Discussion

Natriuretic peptides may be useful as biomarkers of cardioembolic stroke related to paroxysmal atrial fibrillation. Theoretically NT-proBNP measurements may have advantages over BNP due to NT-proBNP increased half-life [4]. In this study, we aimed to determine in a population based study if patients with an initially undetermined stroke etiology that latter had a recurrent stroke etiology in which atrial fibrillation was found had increased levels of BNP at the time of the index stroke. There are some limitations in this study regarding the exams that were done to classify stroke etiology, not all patients did echocardiograms or Holter examinations, nevertheless patients with Transient Ischemic attack or stroke of initially undetermined etiology

in whom atrial fibrillation was detected at the time of subsequent recurrent stroke had had high BNP levels at the time of the first event. Results are in agreement with a recent study, in the general population, that found that elevated plasma Troponin and NT-proBNP concentrations were associated with increased risk of cardioembolic and other nonlacunar ischemic strokes [5]. NT-proBNP level was shown to be a predictor of future development of atrial fibrillation, independent of other risk factors, including echocardiographic parameters in older adults [6]

Conclusion

Patients with Transient Ischemic attack or stroke of initially undetermined etiology in whom atrial fibrillation was detected at the time of subsequent recurrent stroke had had high BNP levels at the time of the first event. BNP may be useful in identification of patients with paroxysmal atrial fibrillation and in the etiological classification of TIA and stroke.

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Discussion

Frequently after an extensive investigation it is not possible to establish an ischemic stroke etiology and strokes end up being classified as undetermined. The diagnostic of an undetermined stroke etiology is many times frustrating for both physician and family. Correctly diagnosing a paroxysmal atrial fibrillation in these circumstances is important because it leads to a change in treatment. Anticoagulants have been shown to be superior to antiplatelets in the secondary prevention of stroke related to atrial fibrillation. To start anticoagulation in all patients with an undetermined stroke etiology is not a solution has it has been shown that for other ischemic strokes the hemorrhagic risk outweighs the benefit [1].

As the currently available methods to diagnose paroxysmal atrial fibrillation have a low sensitivity it is important to consider new ways to diagnose this dysrhythmia in the setting of ischemic stroke. Biomarkers could be an useful alternative. Natriuretic peptides, specifically NT-proBNP a peptide produced by both the hypothalamus and the heart has been shown to be useful in this context. NT-proBNP is produced mainly by the heart, both the atria and ventricles can produce it. It is synthetized and stored in granules. After an episode of atrial fibrillation the pressure and volume overload may lead to the release of the stored granules to the circulation leading to a rise in serum NT-proBNP levels. Studies have shown an increase in NT-proBNP in atrial fibrillation with a return to baseline levels after conversion to sinus rhythm. Therefore NT-proBNP could potentially be used as a biomarker of cardioembolic stroke. As it was also produced by the hypothalamus it was necessary to investigate if levels of NT-proBNP differed in patients with carotid versus vertebro-basilar stroke and also according to the area of stroke infarct. We conducted an initial study that showed that the increase of NT-proBNP levels did no correlate with both of these parameters [2]. We measured NT-proBNP levels in patients with cardioembolic versus non-cardioembolic stroke and reported increased levels in patients with cardioembolic stroke. NT-proBNP had a very good accuracy to diagnose cardioembolic stroke, namely related to atrial fibrillation. From this study cut-off points with a high positive and negative predictive value for the diagnosis of cardioembolic related to atrial fibrillation were determined.

In order to use NT-proBNP as a biomarker of cardioembolic stroke is was necessary to determine its time course after ischemic stroke [3]. Existing information was contradictory. We measured NT-proBNP in the first 24, 48 and 72 hours after stroke onset. Although NT-proBNP serum levels started to decrease at 72 hours, the accuracy of NT-proBNP to diagnose cardioembolic stroke related to atrial fibrillation was similar in the three time points. This decrease of the serum levels after 72 hours may be related to the pattern of release of stored NT-proBNP from the atria.

To fully assess the potential utility of a biomarker, a proof of principle trials should be followed by a prospective validation study in which the diagnostic accuracy necessary for clinical utility is defined a priori. Individual studies tend to optimize the sensitivity and specificity of the cut-off points. A new study was done to validate the established cut-off points and to see if their accuracy was reproduced. In order to do this an adequate sample size was calculated. To ascertain the feasibility of the test, the biomarker validation study was done in the clinical context in which the test would ultimately be used. In the validation study, the values of sensitivity and specificity of the previously established cut-off points were similar to the ones of the original study [4]. The next step was to test the cut-off points in patients with cryptogenic stroke and to see if they were useful to reclassify these ischemic strokes as having a cardioembolic etiology related to paroxysmal atrial fibrillation. Patients with cryptogenic stroke by definition cannot have evidence of significant systolic dysfunction, akinetic or hypokinetic ventricular segments or else they would have been classified as having a cardioembolic stroke etiology. After an extensive investigation including, patients with cryptogenic stroke were followed during one year after stroke onset and did serial Holter ECG monitorings to track atrial fibrillation [4]. There is no ideal method to detect paroxysmal atrial fibrillation. It is possible that episodes of paroxysmal atrial fibrillation may have not been detected. This may partially responsible for a slight decrease of the sensitivity and specificity of the cut-off points, nevertheless the accuracy of the NT-proBNP levels at the time of the stroke onset to diagnose atrial fibrillation was still good.

Although in the validation study, median levels of NT-proBNP in patients with paroxysmal atrial fibrillation were lower than median levels in patients with combined permanent, persistent and paroxysmal atrial fibrillation, the accuracy of NT-proBNP to diagnose paroxysmal atrial fibrillation was good (AUC-0.83). This report of different values of NT-proBNP according to atrial fibrillation was recently reported.

To a biomarker to be useful it needs to have incremental benefit over existing diagnostic and clinical assessment. There are several predictors of paroxysmal atrial fibrillation that are used in clinical practice, including a dilated left atrial on echocardiography, premature atrial and ventricular beats of ECG and the stroke pattern on MRI. However, they have a low accuracy. In our study patients with an initial cryptogenic stroke that were later reclassified as having atrial fibrillation or not did not differ regarding atrial dilatation. Biomarkers such NT-proBNP may be integrated with patient history, clinical assessment, and radiographic data to help to choose patients in whom an extended cardiac monitoring may helpful to unmask the occult paroxysmal atrial fibrillation. In patients with increased levels of NT-proBNP it may be reasonable to recommend longer monitoring with an implantable loop recorder, if the initial 30-day period fails to reveal atrial fibrillation. Thus, biomarkers could serve as an adjunct to clinical and imaging assessment. Also, the NT-proBNP serum measurements can be performed in minutes and is easier to the patient and less expensive than routine workups, such as transthoracic echocardiography or Holter monitoring or other cardiac imaging modalities such as transesophageal echocardiography or cardiac MRI. Also NT-proBNP serum measurements do not have the intra and interobserver variability of the previously mentioned exams.

Currently, it is not possible to indicate that patients with NT-proBNP above a predefined cut-off level should begin anticoagulation. A recent study tried to determine in a retrospectively

design if the effectiveness of warfarin and aspirin in preventing recurrent ischemic stroke or death over 2 years after the initial stroke in noncardioembolic stroke patients differed according to previously determined NT-proBNP levels [5]. This study measured NT-proBNP in serum

collected at the baseline as part of the Antiphospholipid Antibodies and Stroke Study (APASS), a prospective cohort study within the Warfarin–Aspirin Recurrent Stroke Study (WARSS).

Unfortunately, prolonged cardiac rhythm monitoring was not performed in WARSS and it is not known if patients with elevated NT-proBNP levels developed atrial fibrillation during the 2-year follow-up. In this study, in those with NT-proBNP >750 pg/mL, the hazard ratio was 0.30 (95% confidence interval: 0.12–0.84; p=0.021) significantly favoring warfarin over aspirin. It concluded that for secondary stroke prevention, elevated NT-proBNP concentrations could identify a subgroup of ischemic stroke patients without known atrial fibrillation, who could benefit more from anticoagulants than from antiplatelet agents.

Ideally a randomized clinical trial of patients with cryptogenic stroke with serum levels of NT-proBNP above 912 g/ml should be done in order to determine if these patients benefit from anticoagulation versus antiplatelets regarding stroke recurrence.

There are some studies that evaluated the accuracy of BNP instead of NT-proBNP to diagnose cardioembolic stroke. We decided to use NT-proBNP instead of BNP in most of the studies because it has a higher half-life and is reported to be more stable upon freezing of the blood samples. There are no studies that have directly evaluated the accuracy of NT-proBNP versus BNP to diagnose cardioembolic stroke in patients with cryptogenic stroke. Further studies are necessary to compare head to head their diagnostic accuracy.

A main strength of our study was that was done in the clinical context in which it should be applied in a ward during the regular etiological evaluation of stroke etiology. Nevertheless it is necessary to obtain an external validation of the cut-off points. It is necessary to evaluate which was the degree of optimism of the internal validation study. NT-proBNP measured within the first 72 hours after the onset of ischemic stroke may be useful to diagnose atrial fibrillation in patients with strokes initially classified as cryptogenic. NT-proBNP is a relatively inexpensive and widely performed blood test that may be useful as a biomarker of paroxysmal atrial fibrillation in ischemic stroke.

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Conclusions

- NT-proBNP serum levels decrease significantly in the first 72 hours after ischemic stroke. However, the diagnostic accuracy to diagnose a cardioembolic etiology is similar across the first 72 hours
- The cut-off points of 265.5 pg / mL and 912.0 pg / mL of NT-proBNP had a sensitivity, specificity, positive predictive value and negative predictive value in the first study versus validation study of respectively 94.4% vs 100%, 72.9% vs 70.5%, 56.6% vs 59.1%, 97.2% vs 100% versus 55.5% vs 81.8%, 97.9% vs 87.5, 90.9% vs 73.8%, 83.9 vs 91.9% for the diagnosis of ischemic stroke of cardioembolic etiology associated to atrial fibrillation.
- The Area Under the Curve of the Receiver Operating Curve of NT-proBNP for the diagnosis of paroxysmal atrial fibrillation in patients with cryptogenic stroke was good - 0.83, 95% CI (0.73-0.92), the cut-off point of 265.5 pg/mL had a
- sensitivity of 88.2% 95% CI (65.7-96.7%). The cut-off point of 912 pg/mL had a specificity of 88.9% 95% CI (78.8-94.5%).
- In a population based study, patients with higher levels of BNP at the time of an undetermined stroke etiology onset had a higher probability of recurrence related to atrial fibrillation than patients with lower levels of BNP.

Attachments

N-terminal probrain natriuretic peptide as a biomarker of cardioembolic stroke

Ana Catarina Fonseca[1]*, Joaquim Sampaio Matias[2], Teresa Pinho e Melo[1], Filipa Falcão[1], Patrícia Canhão[1], and José M. Ferro[1]

Background and purpose **N-terminal probrain natriuretic peptide, which is mainly produced by the heart, is increased in acute stroke. We aimed to determine if N-terminal probrain natriuretic peptide could be a biomarker for ischemic stroke with a cardioembolic cause.**

Methods **Consecutive sample of acute stroke patients admitted to a Stroke Unit. Ischemic stroke subtype was classified using the TOAST classification. Blood samples were drawn within 72 h after stroke onset. Serum N-terminal probrain natriuretic peptide concentration was measured using an electrochemiluminescence immunoassay. Mean values of N-terminal probrain natriuretic peptide were compared between patients with hemorrhagic stroke vs. ischemic stroke, cardioembolic stroke vs. noncardioembolic stroke, cardioembolic stroke with atrial fibrillation vs. noncardioembolic stroke using *t*-test. Receiver operating characteristic curves were used to test the ability of N-terminal probrain natriuretic peptide values to identify cardioembolic stroke and cardioembolic stroke with atrial fibrillation.**

Results **Ninety-two patients were included (66 with ischemic stroke) with a mean age of 58·6 years. Twenty-eight (42·4%) ischemic strokes had a cardioembolic cause. Mean N-terminal probrain natriuretic peptide values for cardioembolic stroke were significantly higher ($P < 0{\cdot}001$) (491·6; 95% confidence interval 283·7–852·0 pg/ml) than for noncardioembolic ischemic stroke (124·7; 86·3–180·2 pg/ml). The area under the receiver operating characteristic curve for N-terminal probrain natriuretic peptide in cardioembolic stroke was 0·77. The cut-off point with the highest sensitivity and specificity was set at 265·5 pg/ml (71·4% and 73·7% respectively). The area under the curve of N-terminal probrain natriuretic peptide for cardioembolic stroke related to atrial fibrillation was 0·92, cut-off was set at 265·5 pg/ml (sensitivity 94·4%, specificity 72·9%).**

Conclusion **N-terminal probrain natriuretic peptide is a biomarker with a good accuracy to predict ischemic stroke of cardioembolic cause, namely associated with atrial fibrillation.**

Key words: atrial fibrillation, biomarker, cardioembolic, ischemic stroke, NT-proBNP, stroke

Introduction

Even after an extensive clinical investigation, approximately 30–40% of strokes are currently etiologically classified as of undetermined cause (1, 2). It is possible that a fraction of these strokes are due to embolism related to an undetected episode of paroxysmal atrial fibrillation (AF). Several studies show that the current available methods to detect paroxysmal AF have low sensitivity. Although Holter monitoring is superior to ECG in the detection of paroxysmal AF, in a systematic review Holter monitoring (24–72 h) detected new AF in only 4·6% of patients (3). Longer monitoring with different devices can detect AF in 5·7–23% of patients with cryptogenic stroke and a normal ECG or Holter (4–6).

Considering that patients with AF have a high risk of stroke recurrence (7) and that oral anticoagulation in AF is more effective than antiplatelet agents in preventing recurrent stroke (8), it is relevant to develop more accurate ways to detect a cardioembolic origin of stroke.

Brain natriuretic peptide (BNP), which is a neurohormone produced mainly by the heart but also by the brain (9, 10), is increased in acute ischemic stroke (11–15). The BNP is formed after cleavage of a propeptide in two fragments: BNP, which is biologically active and N-terminal probrain natriuretic peptide (NT-proBNP), without biological activity. Both are found in circulation in identical concentrations in normal individuals. Most assays measure NT-proBNP. The NT-proBNP is currently used for diagnostic and prognostic purposes in patients with heart failure and ischemic heart disease. The NT-proBNP is also increased in AF (16). The increase of

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NT-proBNP in acute stroke may have four possible explanations: first, concomitant heart dysfunction (11, 14, 17); second, AF (18); third, response to adrenergic activation (12); and fourth, release from the brain after ischemic injury (13, 19).

We aimed to determine if NT-proBNP could be used as a serum biomarker for ischemic stroke of cardioembolic cause and in ischemic stroke associated with AF.

Materials and methods

Study population

A sample of consecutive acute stroke patients with ischemic (IS) or intracerebral hemorrhage (ICH) admitted from November 2007 to August 2008 to the Stroke Unit of Hospital de Santa Maria, Lisbon, Portugal was used.

Patients with a history of conditions known to increase NT-proBNP (ischemic heart disease, heart failure, heart valve disease, renal failure, cardiomyopathies, pulmonary hypertension, anemia) were excluded. Patients with subarachnoid hemorrhage, cerebral venous thrombosis and transient ischemic attack were also not included in this study.

Clinical protocol

After patient admission from the emergency department, information on demography, vascular risk factors, or previous AF was collected. A brain CT scan, done after hospital admission, was used to classify strokes as hemorrhagic or ischemic. It was repeated 48–72 h after hospital admission to outline the ischemic area. When the ischemic area was not evident in CT scan, a 1·5 T magnetic resonance imaging (MRI; including diffusion-weighted imaging) was performed. All patients had transcranial Doppler and carotid and vertebral duplex scanning.

Etiological workup included, in all patients with IS, complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis and coagulation studies. In patients <55 years old, workup included autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antitrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies.

Transthoracic echocardiogram was performed in all patients. Patients with echocardiographic evidence of heart disease (shortening fraction $<30\%$, valve dysfunction, cardiomyopathies, akinetic ventricular wall regions) were excluded. The following parameters were registered:

- left atria autocontrast
- intracardiac thrombus
- shortening fraction
- patent foramen oval (PFO), and
- akinetic ventricular wall regions.

In patients <55 years old a transesophageal echocardiogram was also performed. To determine the presence of AF at least two ECGs were done during hospital stay, and when the stroke cause remained undetermined a 24 h Holter monitoring was also performed. In patients whose stroke cause remained undetermined, lumbar puncture or cerebral angiography was done. Patients with hemorrhagic stroke had blood analysis, ECG and a transthoracic echocardiogram.

Ischemic stroke causes were classified according to TOAST (20) classifications in five groups; large-artery atherosclerosis, cardioembolic, small-vessel occlusion, stroke of other determined cause and stroke of undetermined cause. For the present study, patients were subdivided in two groups; cardioembolic cause (corresponding to the cardioembolic classification of TOAST) and noncardioembolic cause (all other four groups of the TOAST classification).

Ischemic stroke topography was dichotomized in anterior/carotid territory or posterior/vertebrobasilar territory, accordingly to CT or MRI information. Infarct size was classified according to the ASPECTS scale (21), using CT-scan or MRI information.

To evaluate autonomic nervous system activation three measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were done at eight-hour intervals after patient admission.

In the first 72 h after symptoms onset, four milliliters of blood was drawn from a peripheral vein. Blood samples were immediately centrifugated at 1600 *g* during 15 min. Serum concentration of NT-proBNP was determined by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics®, Mannheim, Germany) (22). A cut-off of NT-proBNP >300 pg/ml has been suggested for heart failure suspicion (23).

The study was approved by the Ethic Committee of the Medicine Faculty of the University of Lisbon and Hospital de Santa Maria. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics

A descriptive statistical analysis of demographic and vascular risk factors of patients with ischemic stroke, hemorrhagic stroke, cardioembolic and noncardioembolic cause was done. Data distribution was evaluated using histograms and a one-sample Kolmogorov–Smirnov test. For normally distributed data, results are presented with mean and standard deviation (SD). For nonnormally distributed data, median and interquartile ranges were defined. For comparison between groups the χ^2-test, Fisher exact test, Mann–whitney test or *t*-test were used as appropriate.

As NT-proBNP values did not follow a normal distribution, a logarithm transformation was done. To compare NT-proBNP values between different timings of blood collection, a one-way analysis of variance was used. *t*-test was used to compare mean values of NT-proBNP between patients with ICH vs. IS, cardioembolic stroke vs. noncardioembolic stroke and cardioembolic stroke related to AF vs. noncardioembolic stroke. Receiver operating characteristic (ROC) curves were

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used to test the ability of NT-proBNP to identify cardioembolic stroke and cardioembolic stroke associated with AF. The area under the curve (AUC) for each ROC curve was determined. Based on the ROC curves, NT-proBNP values with the highest sensitivity and specificity for the diagnosis of cardioembolic stroke and cardioembolic stroke related to AF were determined as well as the corresponding positive and negative predictive values (NPV).

To study the association between NT-proBNP and SBP, DBP and HR, a simple linear regression was calculated. The corresponding regression coefficients with 95% confidence intervals (CI) were determined. To evaluate differences in NT-proBNP values between arterial territories, t-student test was used. To evaluate the association between ischemic area and NT-proBNP value a Kendall correlation analysis was performed. Statistical analyses were done using the SPSS 15.0 for windows. Significance level was set at $P = 0{\cdot}05$.

Results

From November 2007 to August 2008, 202 stroke patients were admitted to the Stroke Unit. Ninety-two patients were included. The main reasons for exclusion were: heart diseases known to increase NT-proBNP (32·7%), subarachnoid hemorrhage (29·1%) and patient admission 72 h after stroke onset (18·2%). Included patients had a mean age of 58·6 (SD$\pm$−14·4) years (64·1% women). Sixty-six (71·7%) patients had an IS and 26 (28·3%) an ICH (Table 1). According to the TOAST classification 28 patients had a cardioembolic cause and 38 were noncardioembolic: 12 (18·2%) large arteries; seven (10·6%) small vessels; five (7·6%) other determined; 14 (21·2%) undetermined. Cardioembolic cause included 18 patients with AF (12 paroxysmal, six permanent) and 10 patients with PFO.

Demographic data, vascular risk factors and vital parameters of patients with cardioembolic IS and noncardioembolic IS were not significantly different (Table 2). The two subgroups of patients with cardioembolic and noncardioembolic cause were not significantly different concerning arterial territory or insular involvement or infarct size evaluated by the ASPECTS scale (Table 2). No intracardiac thrombus or left atria autocontrast were detected during echocardiography.

The NT-proBNP values followed a positively skewed distribution and ranged from 8 to 6378 pg/ml with a median of 177·0 pg/ml. After a logarithm transformation, a new variable was obtained, which followed a normal distribution. In 21 patients (22·8%), blood was collected in the first 24 h, in 62 patients (67·4%) in the first 24–48 h and in nine patients (9·8%) in the first 48–72 h. The NT-proBNP values were not significantly different between different timings of blood collection ($P = 0{\cdot}24$).

The mean value (95% CI) of NT-proBNP in patients with ischemic stroke was 223·18 (157·42–316·40) pg/ml and in patients with hemorrhagic stroke was 133·34 (74·13–239·82) pg/ml. However, this difference was not statistically significant ($P = 0{\cdot}12$).

The mean of NT-proBNP values in patients with ischemic stroke in the carotid artery territory was 275·88 (95% CI 179·47–419·89) pg/ml and in patients with stroke in the vertebrobasilar territory was 138·83 (74·44–259·82) pg/ml. This difference was not statistically significant ($P = 0{\cdot}10$).

No statistically significant association was found between infarct size evaluated by ASPECTS scale and serum values of NT-proBNP ($P = 0{\cdot}11$). No significant linear relationship was found between NT-proBNP values and SBP ($P = 0{\cdot}091$) or DBP ($P = 0{\cdot}26$). A significant linear relationship was found between NT-proBNP values and HR with a regression coefficient of 0·025 pg/ml/bpm, ($P = 0{\cdot}039$).

The mean of NT-proBNP values for cardioembolic stroke was significantly higher ($P < 0{\cdot}001$) (491·6; 95% CI 283·7–852·0 pg/ml) than for noncardioembolic ischemic stroke (124·7; 86·3–180·2 pg/ml) (Fig. 1).

The ROC curve of NT-proBNP values for the diagnosis of cardioembolic stroke had an AUC (95% CI) of 0·77 (0·65–0·89). The cut-off point with the highest sensitivity and specificity was set at 265·5 pg/ml (71·4% and 73·7% respec-

Table 1 Demographic data, vascular risk factors and vital parameters of patients with ischemic and hemorrhagic stroke

	Ischemic stroke ($n = 66$)	Hemorrhagic stroke ($n = 26$)	*P*
Gender, female, (%)	26 (39·4)	7 (26·9)	0·26
Age, years (mean, SD)	60·6 (14·9)	53·6 (11·9)	0·035
Vascular risk factors			
Hypertension (%)	33 (35·9)	15 (16·3)	0·51
Diabetes mellitus (%)	8 (8·7)	4 (4·3)	0·74
Dyslipidemia (%)	20 (22·0)	5 (5·5)	0·27
Smoking (%)	26 (39·4)	9 (34·6)	0·67
Previous AF (%)	7 (10·6)	0 (0)	
Vital parameters			
SBP, mmHg (mean, SD)	140·4 (23·2)	149·5 (28·0)	0·16
DBP, mmHg (mean, SD)	71 (15)	81 (14)	0·01
HR, bpm (mean, SD)	69 (13)	65 (15)	0·33

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SD, standard deviation; AF, atrial fibrillation.

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Table 2 Demographic data, vascular risk factors, stroke characteristics and vital parameters of patients with cardioembolic and noncardioembolic ischemic stroke

	Cardioembolic stroke (n = 28)	Noncardioembolic stroke (n = 38)	P
Gender, male (%)	15 (53·6)	25 (65·8)	0·32
Age, years (mean, SD)	63 (16)	59 (14)	0·24
Vascular risk factors			
Hypertension (%)	12 (18·2)	21 (31·8)	0·32
Diabetes mellitus (%)	3 (4·5)	5 (7·6)	0·99
Dyslipidemia (%)	8 (12·3)	12 (18·5)	0·74
Smoking (%)	16 (57·1)	10 (26·3)	0·60
Anterior arterial territory (%)*	21 (75·0)	27 (77·1)	0·84
Insular involvement	14 (50)	11 (28·9)	0·08
ASPECTS (median)*	6	7	0·19
SBP, mmHg (mean, SD)	139 (22)	141 (24)	0·68
DBP, mmHg (mean, SD)	68 (12)	73 (16)	0·19
HR, bpm (mean, SD)	70 (16)	68 (11)	0·65

*In three patients with small-vessel stroke cause, the second CT did not show any change and a MRI was not performed, therefore the number of patients with a noncardioembolic cause in which topography and location of stroke was evaluated equals 35.SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SD, standard deviation.

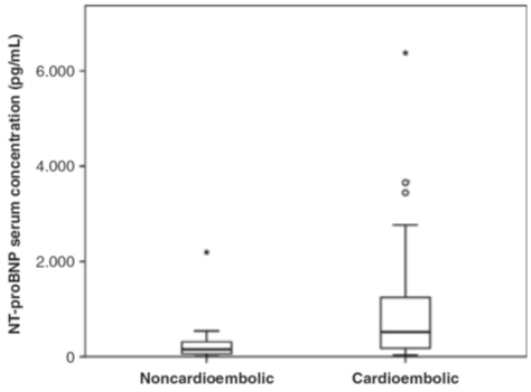

Fig. 1 Serum concentration of NT-proBNP in patients with noncardioembolic and cardioembolic ischemic stroke. Boxplots present median values and interquartile ranges. NT-proBNP, N-terminal probrain natriuretic peptide.

tively). This point had a NPV of 77·8% and a positive predictive value (PPV) of 66·6% (Fig. 2).

The AUC of NT-proBNP obtained for the diagnosis of cardioembolic stroke related to AF was 0·92 (0·86–0·99). This AUC value was higher than the value obtained for the diagnosis of cardioembolic stroke in general (0·92 vs. 0·77). After analysis of the ROC curve for the diagnosis of cardioembolic stroke related to AF a cut-off point of 265·50 pg/ml was determined (sensitivity of 94·4%, specificity of 72·9%, PPV of 56·6% and a NPV of 97·2%). This cut-off point had an extremely high NPV. However, in the context of clinical decisions, it is more important to confirm the diagnosis of cardioembolism as it leads to a change in clinical decision, therefore another cut-off point with a higher PPV was determined. The cut-off point – 912·0 pg/ml had a sensitivity of 55·5%, specificity of 97·9%, PPV of 90·9%, and a NPV of 83·9% (Fig. 2).

Discussion

This study suggests that the increase of NT-proBNP which occurs during stroke has a cardiac origin and may be due to AF. The mean value of NT-proBNP in patients with cardioembolic ischemic stroke was significantly higher than in patients with noncardioembolic ischemic stroke. No significant association was found between stroke territory or infarct size and NT-proBNP values, or between SBP or DBP and NT-proBNP values. The ROC curve for the diagnosis of cardioembolic stroke had an AUC of 0·77 which corresponds to a good ability of NT-proBNP to diagnose cardioembolic stroke. The ROC curve for the diagnosis of cardioembolic stroke associated to AF had an AUC of 0·92 which suggests that NT-proBNP has a very good ability to diagnose ischemic stroke associated to AF, being useful to differentiate it from other etiologies.

Although previous studies had suggested a cardiac cause they had not excluded the presence of confounding variables, which could have caused the increase of NT-proBNP. Namely Montaner (15) and Shibazaki (24) did not exclude the presence of renal failure, heart failure and ischemic heart disease. In our study, to decrease possible confounding factors, patients with known causes of NT-proBNP increase, such as renal failure and heart failure, ischemic heart disease and valvular heart disease, were excluded, using both clinical and echocardiographic evidence. Also, Montaner (15) and Shibazaki (24) did not analyze if this increase could be due to the large infarct area that patients with cardioembolic stroke tend to have (25). In their patients, the increase of NT-proBNP could have been due to a large infarct area, as it is established that NT-proBNP is also produced in the brain (9, 10). In our study, we analyzed this

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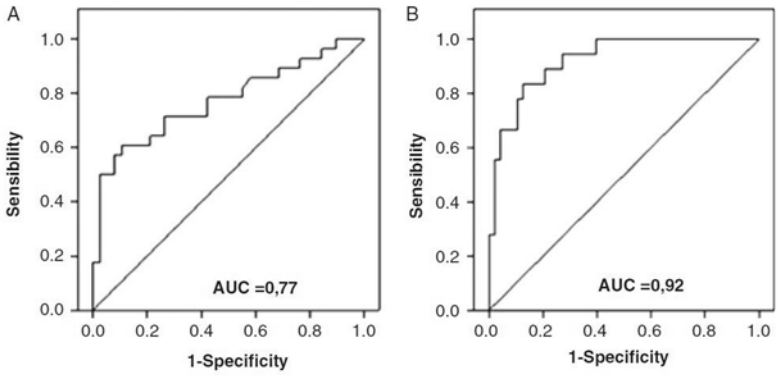

Fig. 2 ROCs curves illustrating the ability of NT-proBNP to identify cardioembolic stroke (a) and cardioembolic stroke associated to AF (b). AUC, area under the curve; NT-proBNP, N-terminal probrain natriuretic peptide; ROC, receiver operating characteristic.

variable and we did not find an association between stroke territory or infarct size and NT-proBNP values.

One recent study (26) suggested that the increase of NT-proBNP in stroke could be due to AF. However, the authors did not exclude patients with heart failure and found that the variable 'congestive heart failure' was significantly higher in the AF group than in non-AF group. Therefore the increase of the NT-proBNP cannot be securely attributed to AF, because heart failure is a cause of NT-proBNP increase (27).

In our study, levels of NT-proBNP were compared between hemorrhagic and ischemic stroke based in the hypothesis that if the increase of NT-proBNP was purely due to a ischemic stroke cause subtype – cardioembolic – and not to other factors such as autonomic activation, there would be higher levels of NT-proBNP in patients with ischemic stroke than in hemorrhagic stroke. In fact, patients with ischemic stroke had higher levels (223·18 (157·42–316·40) pg/ml) than patients with hemorrhagic stroke (33·34 (74·13–239·82) pg/ml). However, this difference was not statistically significant ($P = 0{\cdot}12$) probably due to the modest number of patients included.

When comparing only patients with ischemic stroke, after excluding possible confounding variables, we found that the mean value of NT-proBNP in patients with cardioembolic ischemic stroke was significantly higher than in patients with noncardioembolic ischemic stroke.

Due to the exclusion criteria, the number of possible heart embolic sources was restricted to two: AF and PFO. Patent foramen oval can be a cardioembolic source due to paradoxical embolism or due to the induction of changes in the electrical activity of the left atria leading to atrial arrhythmias, such as paroxysmal AF or atrial flutter (28). It may be therefore able to increase NT-proBNP values.

After analysis of the ROC curve for IS associated to AF, a cut-off point of 265·5 pg/ml with a high sensitivity (94·4%) and a high NPV (97·2%) was determined. The cut-off point of 912·0 pg/ml had a PPV of 90·9%. If this cut-off point is confirmed in another sample it can lead to a high suspicion of AF in patients with stroke of undetermined cause. A NT-proBNP level above this cut-off may help to select patients for prolonged heart rhythm monitoring to detect paroxysmal AF.

In the previously mentioned studies (15, 24, 26), blood was drawn in the first 24 h after stroke onset. In our study, blood was drawn in the first 72 h after stroke onset (although mostly in the first 24–48 h). The option for an enlarged inclusion time was based on the knowledge that a sizable proportion of patients does not go to the hospital in the first 24 h after stroke onset (29). Also, the use of NT-proBNP instead of BNP might be preferred to detect paroxysmal AF because of its prolonged half-life time (30). Available data is unclear regarding the timing when maximum serum concentration of NT-proBNP in IS is achieved. Giannakoulas (12) did not find a statistically significant difference between day one and six after stroke onset. Jensen (31) noticed a peak in the second day, with a progressive decrease in NT-proBNP until day five. Iltumur (17) described highest values of NT-proBNP in the day of stroke onset. In our study no significant difference was found between the different timings of drawing blood samples.

One limitation of our study is the modest number of patients included; nevertheless it obtained some statistically significant results. Other limitations relate to the use, in the majority of patients, of CT instead of MRI to evaluate the infarct area and location.

The study of possible biomarkers of ischemic stroke subtypes may be clinically valuable. Our results suggest that NT-proBNP can be a useful biomarker of certain causes of cardioembolic stroke, namely AF. Our results must be replicated in an independent sample.

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Time course of NT-proBNP levels after acute ischemic stroke

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Background – Studies suggest that N-terminal-pro-brain natriuretic peptide (NT-proBNP) can be a biomarker of cardioembolic stroke. However, the best time to measure it after stroke is unknown. We studied the time course of NT-proBNP in patients with ischemic stroke. *Methods* – Consecutive acute ischemic stroke patients were admitted over 10 months to a Stroke Unit. Stroke type was classified according to TOAST. Blood samples were drawn within 24, 48, and 72 hours after stroke. Friedman test was used to compare NT-proBNP values across the 3 times in all, cardioembolic and non-cardioembolic stroke patients. *Post hoc* analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction. Mann–Whitney test was used to compare median values of NT-proBNP between cardioembolic and non-cardioembolic stroke patients. ROC curves were drawn to determine NT-proBNP accuracy to diagnose cardioembolic stroke at 24, 48, and 72 hours after stroke onset. *Results* – One hundred and one patients were included (29 cardioembolic) with a mean age of 64.5±12.3 years. NT-proBNP values for cardioembolic stroke were significantly higher ($P < 0.001$) than for non-cardioembolic stroke in the 3 time points. NT-proBNP was highest in the first 24–48 h after ischemic stroke and decreased significantly 72 h after stroke onset. The area under the curve for the three time points was similar. *Conclusion* – NT-proBNP levels were highest in the first 2 days after ischemic stroke and declined significantly thereafter. However, the area under the curve for the three time points was similar. The first 72 hours after ischemic stroke have a similar diagnostic accuracy to diagnose cardioembolic stroke.

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Key words: stroke; cardioembolism; etiology; biomarker; NT-ProBNP; time course

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Introduction

The identification of a specific ischemic stroke etiology has important clinical implications in terms of prognosis, recurrence risk, and influences the short-term management and the prescription of secondary prevention interventions.

Particularly, important is the identification of a cardioembolic etiology, namely atrial fibrillation (AF), as studies have shown that anticoagulation is superior to antiplatelet agents for the prevention of recurrent stroke (1). Stroke related to AF tends to be severe, with a mortality rate at the first year of 50%, and is associated to a high recurrence rate (2).

Identifying paroxysmal AF can be particularly difficult as auxiliary complementary examinations can be entirely unremarkable. Literature shows that the current available methods (ECG, routine telemetry during inpatients admission, Holter monitoring, 30-day event monitoring devices) to detect paroxysmal AF have a low sensitivity (3). Therefore, it is plausible that cases of undetected paroxysmal AF may contribute to a large fraction of the approximately 1/3 of strokes which are currently classified as of undetermined etiology (4).

Serum biomarkers may be useful in this context. Recent studies suggest that NT-proBNP may be useful as a serum biomarker of cardioembolic

stroke, namely associated with AF (5–7). NT-proBNP is produced by the atrial and ventricle myocytes in stressful conditions such as hemodynamic overload. Namely, following the hemodynamic effect of atrial fibrillation, granules stored in atrial myocytes are secreted as BNP and NT-proBNP (8), leading to the high levels of NT-proBNP.

However, to use NT-proBNP as a biomarker of cardioembolic stroke, it is necessary to know its short-term kinetic after stroke to determine the best time to measure it. Currently, there is scarce and contradictory data about the best time to measure NT-proBNP after stroke (9, 10).

In this new study, we aimed to characterize NT-proBNP serum levels in the first three days after ischemic stroke.

Methods

Type of study

Observational, prospective study.

Study population

Consecutive sample of ischemic stroke (11) or transient ischemic attack (TIA) (12) patients admitted to a Stroke Unit, from December 2009 to June 2010 and from October 2010 to December 2010. To be included, the patients had to be admitted within 24 hours of stroke onset and have blood collections performed at 24, 48, and 72 hours after stroke onset. Patients were excluded if they had acute renal failure or chronic renal insufficiency (glomerular filtration rate glomerular determined by the equation of Cockcroft-Gault, less than 90 mL/min, hemodialysis or peritoneal dialysis).

Clinical protocol

Patients with ischemic stroke were admitted from the Emergency Department and transferred to the Stroke Unit. In patients who complied with the study criteria, 4 ml of blood was drawn from a peripheral vein, at the first 24 (day 1), 48 (day 2), and 72 hours (day 3) after stroke onset. Blood samples were immediately taken to the Clinical Pathology Department to measure serum levels of NT-proBNP. NT-proBNP levels were measured by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany) (13). A cutoff of NT-proBNP of 300 pg/ml has been suggested for heart failure suspicion (14). The assay has a coefficient of variation in the range of 1.0–6.0%.

During hospital stay, information on demography, vascular risk factors, or previous AF was collected. Etiological workup included complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis, and coagulation studies in all patients. In patients < 55 years old, workup also included autoantibodies, lupus anticoagulant, anti-cardiolipin antibodies, C and S protein, antithrombin III, fibrinogen levels, HIV 1 and 2, Hepatitis B and C serologies. In all patients, a brain-CT or MRI (80.2%) was performed. All patients underwent transcranial Doppler, carotid, and vertebral duplex scanning. Twenty-eight patients (27,7%) had magnetic resonance angiography. In 16 patients (15.8%), digital cerebral angiography was performed. All patients underwent transthoracic echocardiogram. The following parameters were registered: left atria dimensions, ejection fraction, presence of left atrial spontaneous echo contrast, intracardiac thrombus, patency of patent foramen oval (PFO), and hypokinetic/akinetic ventricular segments. In patients < 55 years old, a transesophageal echocardiogram was also performed. To detect the presence of AF, at least two ECGs and one 24-hour Holter monitoring were performed during hospital stay. Stroke etiological classification was performed using the TOAST criteria (15). Stroke subtypes were further grouped in cardioembolic and non-cardioembolic (which included the undetermined).

The study was approved by the Ethic Committee of Hospital de Santa Maria, Lisbon, Portugal. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics

A descriptive statistical analysis of demographic and vascular risk factors of patients with ischemic stroke, cardioembolic, and non-cardioembolic cause was performed. Data distribution was evaluated using histograms and a one sample Kolmogorov–Smirnov test. For normally distributed data, results were presented with mean and standard deviation (SD). For non-normally distributed data, median and interquartile ranges were defined. For comparison between groups, the chi-squared test, Fisher exact test, Mann–Whitney test, or T-test were used as appropriate.

To compare NT-proBNP values between the 3 different timings of blood collection, Friedman test was used. This was performed for all ischemic stroke patients and for those with cardioembolic

and non-cardioembolic stroke. *Post hoc* analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction.

Mann–Whitney test was used to compare the median values of N-BNP between groups of patients with cardioembolic stroke versus non-cardioembolic stroke.

Receiver operating curves and the area under the curve to determine the accuracy of NT-proBNP to diagnose cardioembolic stroke were determined at each time point.

Statistical analyses were performed using the *SPSS 19.0* for windows. Significance level was set at $P = 0.05$.

Results

Between December 2009–June 2010 and October 2010–December 2010, 160 patients with ischemic stroke or TIA were admitted to the Stroke Unit. One hundred and one patients were included in the study (93 with stroke, 8 with TIA). Main reasons for patients' exclusion from the study were as follows: admission more than 24 hours after stroke onset ($n = 26$), unavailability of 3 measurements ($n = 14$), less than 3 days of hospital stay ($n = 10$), and renal failure ($n = 1$).

Included patients had a mean age of 64.5 years with a standard deviation (SD) of 12.3. Minimum age was 31 years, and the maximum age was 86. Table 1 shows the characteristics of included patients. Classification of stroke etiology according to TOAST criteria is presented in Table 1. The main stroke etiology was undetermined followed by cardioembolic etiology. Other determined etiology was due to artery dissection in two cases and hypercoagulable conditions in four cases (immunoglobulins infusion 1, iron deficiency anemia 1, antiphospholipid syndrome 1, and thrombocytosis 1).

Three hundred and three NT-proBNP determinations were performed. The NT-proBNP serum determinations were performed at exactly the following time points: mean (SD) 23 h 02 min (1 h 51 min), 46 h 50 min (2 h 40 min), and 71 h 17 min (2 h 42 min). NT-proBNP values did not follow a normal distribution across the three time points (24, 48, 72 h). They had a positively skewed distribution. Median values of NT-proBNP were highest in the first 24 hours after ischemic stroke and decreased in the following time points. The difference of NT-proBNP values across the 3 time points was statistically significant ($P < 0.001$). When we analyzed individual differences between the specific time points, there was no significant difference in NT-proBNP values at day 1 vs day 2 ($P = 0.34$). Values of NT-proBNP were different at day 2 vs day 3 ($P < 0.001$).

Table 1 Characteristics of the included patients

	All	Cardioembolic	Non-cardioembolic	*P*
Patients (n)	101	29	72	
Age, years (mean ± SD)	64.5 ± 12.3	65.4 ± 12.2	63.5 ± 12.6	0.53
Gender, Female (*n*, %)	42 (41.6)	16 (55.2)	25 (34.7)	0.06
Hypertension (*n*, %)	71 (70.3)	19 (65.5)	52 (72.2)	0.51
Diabetes mellitus (%)	25 (24.8)	6 (21)	19 (26.4)	0.55
Dyslipidaemia (*n*, %)	33 (32.7)	8 (27.6)	25 (34.7)	0.49
Anemia (*n*, %)	11 (10.9)	6 (20.7)	5 (6.9)	0.07
ACEI or ARB (*n*, %)	50 (49.5)	13 (44.8)	37 (51.4)	0.55
Beta-blockers (*n*, %)	25 (24.8)	9 (31.0)	16 (22.2)	0.35
Diuretics (*n*, %)	31 (30.7)	8 (27.6)	23 (31.9)	0.67
Systolic dysfunction (*n*, %)	6 (6.0)	4 (13.8)	2 (2.8)	0.06
Diastolic dysfunction ($n = 76$)	32 (42.1)	5 (23.8)	27 (49.1)	0.05
NT-proBNP day 1				
Median	392.0	1203.0	170.5	<0.001
1st quartile	109.0	827.0	66.5	
3rd quartile	1077.5	2109.5	510.8	
NT-proBNP day 2				
Median	351	1607.0	177.5	<0.001
1st quartile	97	684.5	72.0	
3rd quartile	1132	2947.0	494.8	
NT-proBNP day 3				
Median	230	1380.0	144.0	<0.001
1st quartile	98	579.5	42.8	
3rd quartile	975	2390.0	288.8	
Etiology				
Undetermined	34			
Cardioembolic	29			
Atrial fibrillation	(18)			
Hypokinetic VS	(2)[†]			
Dil. Cardiomyop	(2)			
Atrial flutter	(1)			
Endocarditis	(2)			
PFO	(2)			
PFO/ASA	(1)			
Left atria smoke	(1)			
Large vessels	28			
Intracranial	(12)			
Extracranial	(16)			
Other determined	6			
Small vessels	4			

SD, standard deviation; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; Hypokinetic VS, hypokinetic ventricular segment; Dil. Cardiomyop, dilated cardiomyopathy; PFO, patent foramen ovale; ASA, atrial septal aneurysm.

NT-proBNP values in patients with cardioembolic stroke ($n = 29$) were higher than in patients with non-cardioembolic stroke ($n = 72$) in the three time points ($P < 0.001$); (Table 1).

The same pattern of NT-proBNP values kinetic was observed in patients with cardioembolic and non-cardioembolic stroke. NT-proBNP values were not significantly different in the first 2 days. However, in the third day, there was a statically

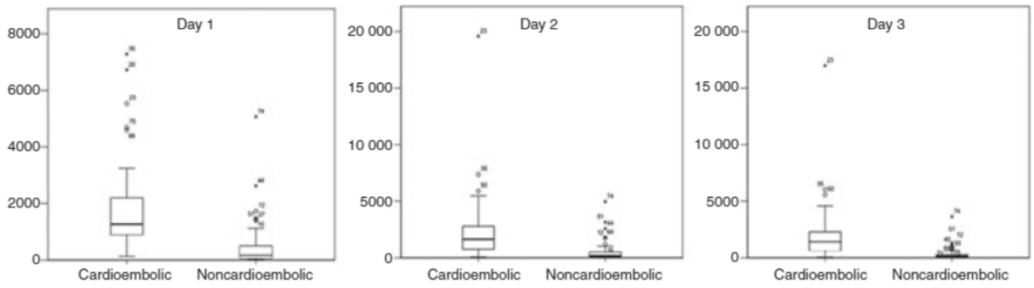
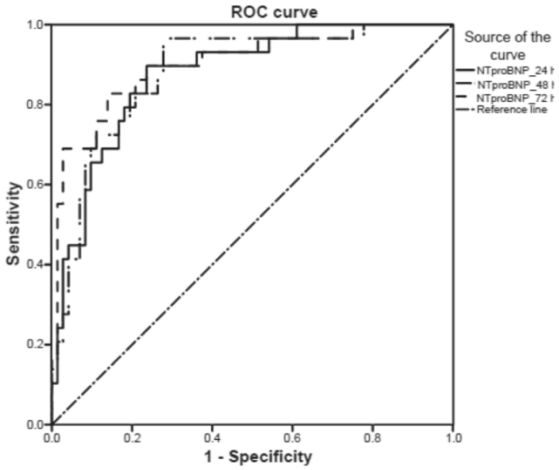


Figure 1. Serum levels of NT-proBNP in patients with cardioembolic (n = 72) and non-cardioembolic stroke (n = 29) in days 1–3. NT-proBNP values expressed in pg/mL.

significant decrease, cardioembolic day 1 vs day 2 vs day 3 $P = 0.035$, day 1 vs day 2 $P = 0.42$, day 2 vs day 3 $P = 0.023$, non-cardioembolic day 1 vs day 2 vs day 3 $P < 0.001$, day 1 vs day 2 $P = 0.10$, day 2 vs day 3 $P < 0.001$. (Fig 1)

The receiver operating curves of NT-proBNP at 24, 48, and 72 hours for the diagnosis of cardioembolic stroke had a similar very good area under the curve (AUC) (Fig 2). Despite the significant difference in the absolute values of the 3 times points, the area under the curve of NT-proBNP at 72 hours was not inferior to the value at 24–48 hours. The number of patients with levels of NT-proBNP above the prespecified cutoff point with a high sensitivity for the diagnosis of cardioembolic stroke of 265.5 pg/mL was similar in the three time points (Fig 3) (5). The areas under the curve values were for day 1 – 0.88 (95% CI 0.81–0.95), day 2 – 0.88 (95% CI 0.81–0.95), day 3 – 0.90 (95% CI 0.83–0.97), $P < 0.001$.



	Area under the curve (c-statistic)	*P*-value	95% Confidence Interval
NTproBNP 24 h	0.876	<0.0001	0.806–0.947
NTproBNP 48 h	0.882	<0.0001	0.811–0.954
NTproBNP 72 h	0.902	<0.0001	0.832–0.972

Figure 2. Receiver operating characteristic curves and respective areas under the curve (AUC) regarding the accuracy of NT-proBNP to diagnose cardioembolic stroke at 24, 48, and 72 hours after cardioembolic stroke. Areas under the curve values can vary between 0 and 1. A value of 0.5 means that the test is useless, 1 means that the test has a perfect diagnostic accuracy.

Discussion

In all included stroke patients and in those with cardioembolic and non-cardioembolic stroke (including undetermined causes), values of NT-proBNP were highest at 24 and 48 hours after ischemic stroke with no statistically significant difference between these two time points and had a statistically significant reduction 72 h after stroke onset. However, the area under the curve for the three time points showed similar diagnostic accuracy, which suggests that measurements of NT-proBNP in first 72 hours after ischemic stroke are equally useful.

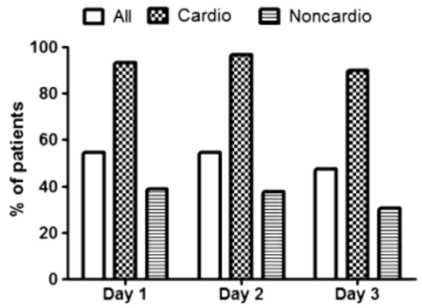


Figure 3. Percentage of all patients, cardioembolic, and non-cardioembolic patients (including undetermined causes) with NT-proBNP values above the cutoff 265.5 pg/mL at day 1, day 2, and day 3.

Previously, two reports in the literature analyzed the variation of NT-proBNP in the first days after ischemic stroke (9, 10). Iltumur (10) measured NT-proBNP levels in 57 patients at days 1, 3, 5, and 10 after ischemic stroke. NT-proBNP levels were highest in the first day after ischemic stroke and declined significantly from day 3 onwards. However, there was no information about day 2 after stroke, and individual stroke etiologies were not analyzed. Jensen (9) measured NT-proBNP levels in 250 patients at days 1, 2, 3, 4, and 5 after ischemic stroke. He found highest levels at day 2, with a significant difference between day 1 and day 2. Thereafter, there was a progressive decreased until day 5. However, there was no information regarding the specific etiologies of stroke in these patients or the diagnostic accuracy of NT-proBNP in different time points. Patients with atrial fibrillation were excluded from the study.

In previous reports that studied NT-proBNP or BNP as a possible biomarker of cardioembolic stroke, measurements have been performed during the first 24 hours after ischemic stroke (6, 7, 16). The knowledge of an extended time window (72 hours) for the determination of NT-proBNP might be useful as a significant proportion of patients does not go to the hospital in the first 24 h after stroke onset (17). This was a major cause of exclusion of patients in our cohort. The possibility of an increased time window for NT-proBNP measurements is an important possible advantage of the use of NT-proBNP instead of BNP in this setting, as NT-pro BNP is known to have an increased half-life (18). This extended time window of 72 hours can also have an advantage over the user of D-dimers for the diagnosis of cardioembolic stroke, which were found to be potentially useful for the diagnosis of cardioembolic stroke in the first 12 hours after ischemic stroke (19).

One limitation of our study was that only three time points with intervals of 24 hours were analyzed, eventually if shorter intervals were chosen they would be even more informative.

The proportion of undetermined stroke etiology in our cohort is similar to the proportion stated in other studies – a range from 30 to 40% (20, 21). Although 20 (19.8%) patients presented with a clinical lacunar syndrome, in only 4 patients, it was due to small vessels disease. This was due to a high rate of use of DWI-MRI. DWI-MRI improves the accuracy of the subtype diagnosis of stroke (22). Most cases of clinical lacunar syndromes not associated with small vessels disease were due to large artery or cardiogenic embolic stroke.

Our observation of higher levels of NT-proBNP in patients with cardioembolic stroke than in non-cardioembolic stroke is in accordance with previous studies. The finding of a statistically significant difference in NT-proBNP across the 3 times points and the very good value of accuracy to diagnose cardioembolic stroke reinforces the idea that NT-proBNP may be used as a biomarker of cardioembolic stroke. Serum biomarkers may have an important role in the diagnosis of paroxysmal AF, even if ongoing trials of implantable event detectors have successful results (23). To obtain NT-proBNP serum measurements, it is necessary to perform a venipuncture, but this is a less invasive procedure than the implantation of an event detector. NT-proBNP measurements are also less expensive and are widely available in most wards and emergency departments.

They could potentially be incorporated in a subclassification of patients with undetermined etiology and help in the stratification of patients that could benefit the most from long-term heart rhythm monitoring.

Conclusion

NT-proBNP levels were highest 24–48 hours after ischemic stroke and started to decline thereafter. However, the diagnostic accuracy of NT-proBNP to diagnose cardioembolic stroke was similar and very good in the first 72 hours after stroke onset. Our study reinforces that BNP may be useful in the identification of patients with cardioembolic stroke and in the etiological classification of TIA and stroke.

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Conflict of interest

The authors have no conflict of interest to disclose.

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N-terminal pro-brain natriuretic peptide shows diagnostic accuracy for detecting atrial fibrillation in cryptogenic stroke patients

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Background **Diagnosing paroxysmal atrial fibrillation in patients with stroke can be difficult. We aimed to determine if *N*-terminal pro-brain natriuretic peptide can help identify paroxysmal atrial fibrillation in cryptogenic stroke.**

Methods and results **Among 264 ischemic stroke patients, serum levels of *N*-terminal pro-brain natriuretic peptide were measured within 72 h of stroke onset. In cryptogenic stroke patients, 24-h Holter monitoring was used to look for paroxysmal atrial fibrillation within the first week and also three- and six-months after admission. First, patients with a defined etiology were used to construct a receiver operating characteristic curve for the diagnosis of atrial fibrillation. From this curve, the sensitivity and specificity of preestablished cutoff points for the diagnosis of atrial fibrillation were calculated. A logistic regression was performed to assess the independent relationship of the logarithm of *N*-terminal pro-brain natriuretic peptide levels with atrial fibrillation. The cutoff points were then evaluated in patients with cryptogenic stroke.**

Results **One hundred eighty-four patients had a specific stroke etiology. Fifty-five patients had atrial fibrillation. Using multivariate analysis, the logarithm of *N*-terminal pro-brain natriuretic peptide levels was independently associated with atrial fibrillation. The area under the receiver operating characteristic curve of *N*-terminal pro-brain natriuretic peptide for the diagnosis of atrial fibrillation was 0·91 (95% confidence interval 0·87–0·95). The cutoff point of 265·5 pg/ml had a sensitivity of 100% and specificity of 70·5% for the diagnosis of atrial fibrillation. The cutoff point of 912 pg/ml had a sensitivity of 81·8% and a specificity of 87·5%. Eighty patients had a cryptogenic stroke. In 17, paroxysmal atrial fibrillation was found during follow-up. In these patients, the area under the curve for the diagnosis of paroxysmal atrial fibrillation was 0·83. The cutoff point of 265·5 had a sensitivity of 88·2% and a specificity of 61·9%. The cutoff point of 912 pg/ml had a sensitivity of 47·1% and a specificity of 88·9%.**

Conclusion ***N*-terminal pro-brain natriuretic peptide has good accuracy in predicting the presence of paroxysmal atrial fibrillation in patients with cryptogenic stroke and can help to identify these patients.**

Key words: atrial fibrillation, biomarker, cryptogenic, diagnosis, NT-proBNP, stroke

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Introduction

Stroke related to atrial fibrillation (AF) tends to be severe, with high recurrence and mortality rates (1). Identification of AF in patients with stroke is consequently of paramount importance and has therapeutic implications. AF is usually classified as permanent, persistent, or paroxysmal. Paroxysmal AF (pAF) is reported to have the same risk as persistent or permanent AF to cause ischemic strokes (2).

However, identifying pAF can be difficult. Although it is frequently related to structural heart disease, about 45% of patients with pAF have no echocardiographically detectable heart disease (3). The currently available electrophysiological methods [echocardiogram (ECG), routine telemetry during inpatient admission, Holter monitoring, 30-day event monitoring devices] for detecting pAF all have a low sensitivity (4). If diagnostic tests fail to identify pAF, stroke etiology may be incorrectly classified as cryptogenic (undetermined etiology) (5).

Alternative ways to detect pAF in cryptogenic stroke should be considered. Recent studies suggest that *N*-terminal pro-brain natriuretic peptide (NT-proBNP), a peptide produced by the heart, may be useful to identify cardioembolic stroke associated with AF (6–8). In a previous study, NT-proBNP had good accuracy in predicting ischemic stroke of cardioembolic cause associated with AF. Two cutoff points – 265·5 pg/ml and 912·0 pg/ml, associated with a high negative and high positive predictive value for the diagnosis of AF (97·2% and 90·9%, respectively) – were obtained (6).

In this study, we aimed to determine if NT-proBNP could identify pAF in patients with strokes initially classified as cryptogenic and validate the previous cutoff values of NT-proBNP levels for the diagnosis of AF.

Methods

Study type

This was an observational, prospective cohort study.

Study population

The study population consisted of consecutive patients admitted to the stroke unit of a neurology department from December 2009 to June 2010, from September 2010 to December 2010, and from April 2011 to March 2012. In order to be included, patients had to have an ischemic stroke (according to the World Health Organization criteria) (9) or transient ischemic attack (TIA) (10) and be admitted to the stroke unit within 72 h of stroke onset. Patients were excluded if they had acute renal failure or chronic renal insufficiency (glomerular filtration rate determined by the

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Cockcroft–Gault equation less than 90 ml/min, hemodialysis, or peritoneal dialysis). Renal insufficiency is a cause of NT-proBNP increase.

Sample size calculation

Taking into account the information from a previous publication (6), the mean of NT-proBNP levels in patients with a cardioembolic ischemic stroke was 491·6 pg/ml with a standard deviation (SD) of 420·96 ($n = 28$), and the mean in patients with noncardioembolic stroke was 124·7 pg/ml with a SD of 69·55 ($n = 38$). With a power of 95% and an alpha of 0·05 to show a statistically significant difference between groups, a total sample size of 40 patients with undetermined stroke etiology was needed. Assuming a percentage of cryptogenic strokes of 16%, a cohort of 250 patients was needed. To validate the cutoff values of NT-proBNP levels in patients with a defined stroke etiology, taking into account the previously evaluated sensitivity of 94·4% of the cutoff value of 265·5 pg/ml and the specificity of 97% of the cutoff value of 912·0 pg/ml (6), in a population where the prevalence of AF is 0·30, with a required 95% lower confidence limit >0·8 at 0·95 probability, 50 patients and 116 controls were required (total 166 patients with a defined stroke etiology) (11).

Clinical protocol

Patients with ischemic stroke or TIA were admitted to the stroke unit. In patients who met the study criteria, four-milliliters of blood were drawn from a peripheral vein within 72 h of stroke onset. Blood samples were immediately taken to the clinical pathology department. Blood samples were centrifuged at 1600 g for 15 min. NT-proBNP levels were measured by an electrochemiluminescence assay using the Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). A full description of the method and its evaluation is available elsewhere (12). The assay has a coefficient of variation in the range of 1·0–6·0%.

Information on demography, vascular risk factors, previous AF, and National Institutes of Health Stroke Scale (NIHSS) score on admission (13) was collected. Etiological workup included, in all patients, a complete blood count, erythrocyte sedimentation rate, hepatic and renal function, glucose and lipid levels, protein electrophoresis, and coagulation studies. In patients <55 years old, workup also included autoantibodies, lupus anticoagulant, anticardiolipin antibodies, C and S protein, antithrombin III, fibrinogen levels, HIV 1 and 2, and hepatitis B and C serologies. In all patients, brain computed tomography or magnetic resonance imaging (71% MRI), transcranial Doppler, carotid and vertebral duplex scanning, and transthoracic echocardiographic examination (M-mode, two-dimensional, and Doppler study) were performed. The following parameters were registered: atrial dimensions (atrial dilatation was defined as an end-systolic diameter > 40 mm) (14), left atrial spontaneous echo contrast, intracardiac thrombus, left ventricular ejection fraction (LVEF), persistence of patent foramen ovale, hypokinetic ventricular wall regions, diastolic dysfunction (15), and left ventricular hypertrophy. Systolic dysfunction was defined as an LVEF < 50%. In all patients <55 years old (18·7% of total patients), contrast transcranial Doppler with agitated saline was done to look for a right–left shunt, and transesophageal echocardiography was performed. To detect pAF, at least two ECGs and one 24-h Holter monitoring were performed within the first week of the presenting event. Stroke etiological classification was done by stroke neurologists using the Trial of Org 10172 in Acute Stroke Treatment criteria, blind to NT-proBNP determinations (16). If patients had a history of AF, stroke was considered to be cardioembolic if no other finding was disclosed. Patients with a cryptogenic stroke were followed as outpatients. During follow-up, in all patients, after hospital discharge, independently of NT-proBNP, serial 24-h Holter monitoring was performed within three- and six-months (if the previous monitoring was unremarkable) to look for pAF and was used as reference standard. AF was defined as a dysrhythmia with at least 30 continuous seconds (17) with no detectable P waves and no other diagnosis. The study was approved by the ethics committee of our hospital. A signed informed consent was obtained from the patient or from a relative or legal representative.

Statistics

A descriptive statistical analysis of demographic and vascular risk factors of all patients with ischemic stroke, AF, and nonatrial fibrillation was performed. Data distribution was evaluated using histograms and a one-sample Kolmogorov–Smirnov test. For normally distributed data, results were presented with mean and SD. For nonnormally distributed data, median and interquartile ranges were defined. For comparison between groups, the chi-square test, Fisher's exact test, Mann–Whitney test or *t*-test were used as appropriate.

First, patients with a defined etiology were used to construct a receiver operating characteristic (ROC) curve to determine the accuracy of NT-proBNP for the diagnosis of AF. In this case, the gold standard for the diagnosis of AF was a known and established diagnosis based on a previous ECG or Holter. From this curve, the area under the curve (AUC; c-statistics) and the sensitivity and specificity – with the respective confidence intervals of the established cutoff points – were calculated (6). The cutoff points of 265·5 and 912·0 pg/ml of NT-proBNP were chosen in a previous study for their high sensitivity (94·4%) and specificity (97·9%), respectively (6). A multivariate analysis was performed, using a logistic regression model, to assess the independent relationship of log NT-proBNP with AF, controlling for previously known confounders – age, sex, atrial dilatation, left ventricular hypertrophy, and systolic dysfunction. Variables were included in the logistic regression model as predictors based on prior knowledge and also based on the previous study of the association between the different variables and outcome or exposure. Results are presented as odds ratios and 95% confidence intervals (CIs). In a second phase, the cutoff points previously established were evaluated in patients with cryptogenic stroke for the diagnosis of pAF.

Statistical analyses were done using SPSS 19·0 (IBM Corporation, Armonk, NY, USA) and SAS 9·2 for Windows. Significance level was set at $P = 0·05$.

This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) statement procedures (18).

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Results

Two hundred sixty-four patients were included, with a mean age of 63·8 (SD 12·2) years (Fig. 1). Twenty-five patients had a TIA (9·5%). In 184 patients, a specific stroke etiology was established. The specific stroke etiologies were: 94 cardioembolic (51·1%), 57 large-vessel disease (31·0%), 17 small-vessel disease (9·2%), and 6 undetermined/more than one possible etiology (3·3%). Among cardioembolic strokes, 52 were due to AF, 4 patent foramen ovale plus atrial septum aneurysm, 19 patent foramen ovale, 11 hypokinetic/akinetic left ventricular segments, 3 endocarditis, 1 mitral valve prolapse, 2 mechanical valvular prosthesis, 1 dilated cardiomyopathy, and 1 heart tumor. Three of the six patients with more than a possible stroke etiology had both AF and ipsilateral >50% carotid stenosis. A total of 55 patients had AF. Patients with AF were more frequently older and female, had atrial dilatation, and had higher admission NIHSS scores (Table 1). Patients without AF were more frequently smokers.

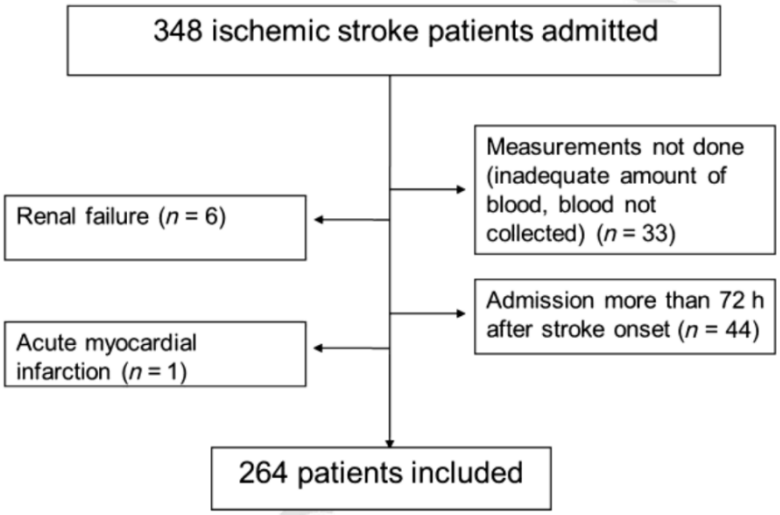

Fig. 1 Fluxogram of included patients.

Table 1 Characteristics of patients with a known stroke etiology, with or without atrial fibrillation

	AF (*n* = 55)	No AF (*n* = 129)	*P* value
NT-proBNP (pg/dl), median (IQR)	1777 (2710)	138 (332)	<0·0001
Transient ischemic attack, *n* (%)	2 (3·6)	18 (14·0)	0·04
Age (years), mean (SD)	70·3 (7·8)	58·6 (12·7)	<0·0001
Female, *n* (%)	31 (56·4)	48 (37·2)	0·02
Hypertension, *n* (%)	36 (65·5)	91 (70·5)	0·49
Diabetes, *n* (%)	10 (18·2)	37 (28·7)	0·14
Dyslipidemia, *n* (%)	29 (52·7)	54 (41·9)	0·18
Current smoker, *n* (%)	4 (7·3)	39 (30·2)	0·001
Previous myocardial infarction or angina, *n* (%)	12 (21·8)	18 (14·0)	0·19
Previous stroke or transient ischemic attack, *n* (%)	21 (38·2)	30 (23·3)	0·04
Hemoglobin (g/dl), mean (SD)	14·0 (1·7)	13·9 (1·8)	0·71
Weight (kg), median (IQR)	74·1 (19·5)	75·0 (14·6)	0·22
Angiotensin-converting enzyme inhibitors, *n* (%)	16 (29·1)	33 (25·6)	0·62
Angiotensin receptor blockers, *n* (%)	11 (20·0)	22 (17·1)	0·63
Beta-blocker, *n* (%)	23 (41·8)	23 (17·8)	0·001
Admission NIHSS score, median (IQR)	13 (12)	4 (8)	<0·0001
Anterior circulation stroke, *n* (%)	53 (96·4)	89 (70·6)	<0·0001
Atrial dilatation, *n* (%)	42 (76·4)	31 (24·0)	<0·0001
Left ventricular hypertrophy, *n* (%)	21 (38·2)	33 (25·6)	0·09
Systolic dysfunction, *n* (%)*	10 (18·2)	14 (10·9)	0·18
Diastolic dysfunction, *n* (%) (out of 115)	6 (21·4)	47 (54·0)	0·003
Segmental left ventricular hypokinesia or akinesia, *n* (%)	10 (18·2)	17 (13·2)	0·38

*Defined as a left ventricular ejection fraction <50%. AF, atrial fibrillation; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; IQR, interquartile range; SD, standard deviation; NIHSS, National Institutes of Health Stroke Scale.

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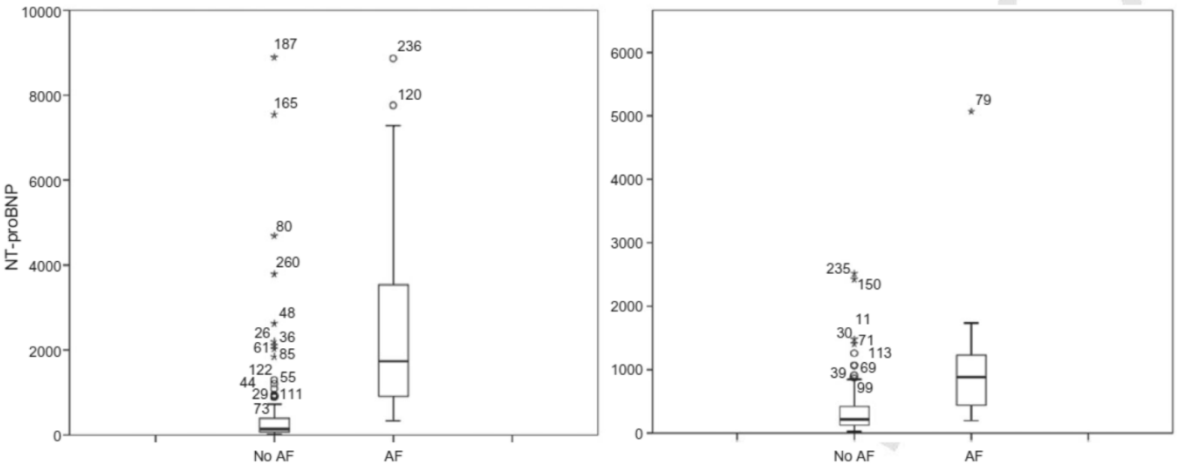

Fig. 2 *N*-terminal pro-brain natriuretic peptide (NT-proBNP) levels (pg/ml) in patients with a specific stroke etiology, with or without atrial fibrillation (AF) (left) and in patients with initial cryptogenic stroke with or without later paroxysmal AF (right).

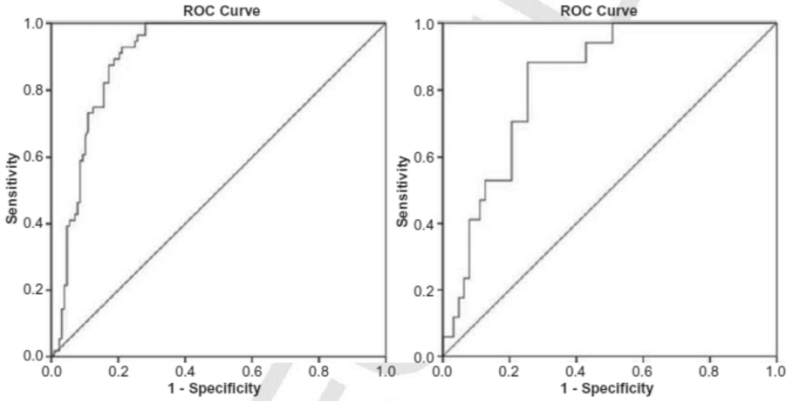

Fig. 3 Receiver operating characteristic (ROC) curve of *N*-terminal pro-brain natriuretic peptide for the diagnosis of atrial fibrillation (AF) in patients with known stroke etiology (left) and for the diagnosis of paroxysmal AF in patients with an initial cryptogenic stroke (right).

Table 2 Correlation of NT-proBNP values with vascular risk factors and echocardiographic parameters

	Phi coefficient	*P* value
Gender	0·111	0·131
Hypertension	0·066	0·370
Diabetes mellitus	0·031	0·674
Atrial fibrillation	0·646	<0·001
Atrial enlargement	0·529	<0·001
Left ventricular systolic dysfunction	0·319	<0·001
Left ventricular hypertrophy	0·232	0·003

NT-proBNP cutoff 265·5 pg/ml. NT-proBNP, *N*-terminal pro-brain natriuretic peptide.

NT-proBNP levels followed a right-skewed distribution. Levels of NT-proBNP were higher in patients with AF than in patients without AF (Fig. 2).

Correlation of values of NT-proBNP levels with vascular risk factors and echocardiographic parameters is shown in Table 2.

Using multivariate analysis, log NT-proBNP was independently associated with AF [odds ratio 2·65, 95% CI 1·57–4·45; $P < 0·0001$) after adjusting for age, sex, left ventricular hypertrophy, atrial dilatation, and systolic dysfunction. Adding admission NIHSS scores to the model did not change the significance of the association between log NT-proBNP and AF. Among patients with a specific stroke etiology, the AUC of the ROC curve of NT-proBNP for the diagnosis of AF was excellent (−0·91; 95% CI 0·87–0·95) (Fig. 3). An AUC of 1 means that the test has a perfect diagnostic accuracy; 0·5 means that it is useless. The previously defined cutoff of 265·5 pg/ml had a sensitivity of 100% (95% CI 93·5–100%), a specificity of 70·5% (95% CI 62·2–77·7%), a positive predictive value of 59·1% (95% CI 48·5–69·2%), and a negative predictive value of 100% (95% CI 96·0–100%). In this study, the cutoff value of 304 pg/ml had the same sensitivity (100%; 95% CI 93·5–100%) with a higher specificity of 71·3% (95% CI 62·7–78·9%), positive predictive value of 59·8% (95% CI 49·0–69·9%), and negative predictive value of 100% (95% CI 96–100%). The cutoff point of 912 pg/ml had a sensitivity of 81·8% (95% CI 69·7–89·8%), a specificity of 87·5% (95% CI 80·8–92·2%), a posi-

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Defined stroke etiology

265.5 pg/ml

	AF	No AF
>	55	38
<	0	91

912.0 pg/ml

	AF	No AF
>	45	16
<	10	113

Cryptogenic stroke

265.5 pg/ml

	AF	No AF
>	15	24
<	2	39

912.0 pg/ml

	AF	No AF
>	8	7
<	9	56

Fig. 4 Distribution of patients according to the defined cutoff point and the presence or absence of atrial fibrillation (AF).

Table 3 Characteristics of patients with initial cryptogenic stroke

	AF (*n* = 17)	No AF (*n* = 63)	*P*-value
NT-proBNP (pg/dl), median (IQR)	883 (817)	214 (367)	<0·0001
Transient ischemic attack, *n* (%)	0 (0)	5 (7·9)	0·58
Age (years), median (IQR)	74 (7)	68 (15)	0·006
Female, *n* (%)	13 (76·5)	25 (39·7)	0·12
Hypertension, *n* (%)	12 (70·6)	45 (71·4)	0·99
Diabetes, *n* (%)	4 (23·5)	19 (30·2)	0·77
Dyslipidemia, *n* (%)	8 (47·1)	18 (28·6)	0·16
Current smoker, *n* (%)	0 (0)	7 (11·1)	0·34
Previous myocardial infarction or angina, *n* (%)	2 (11·8)	7 (11·1)	0·99
Previous stroke or transient ischemic attack, *n* (%)	3 (17·6)	15 (23·8)	0·75
Hemoglobin (g/dl), mean (SD)	13·4 (1·0)	13·7 (1·4)	0·42
Weight (kg), median (IQR)	70 (11·9)	74 (12·5)	0·22
Angiotensin-converting enzyme inhibitors, *n* (%)	6 (35·3)	11 (17·5)	0·18
Angiotensin receptor blockers, *n* (%)	4 (23·5)	15 (23·8)	0·99
Beta-blocker, *n* (%)	7 (41·2)	12 (19·0)	0·10
Admission NIHSS score, median (IQR)	13 (11)	7 (8)	0·04
Anterior circulation stroke, *n* (%)	16 (94·1)	48 (76·2)	0·17
Atrial dilatation, *n* (%)	10 (58·8)	21 (33·3)	0·06
Left ventricular hypertrophy, *n* (%)	4 (23·5)	24 (38·1)	0·26
Systolic dysfunction, *n* (%)*	1 (5·9)	0 (0)	0·21
Diastolic dysfunction, *n* (%) (*n* = 60)	7 (53·8)	27 (57·4)	0·82

*Defined as a left ventricular ejection fraction <50%. AF, atrial fibrillation; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; IQR, interquartile range; SD, standard deviation; NIHSS, National Institutes of Health Stroke Scale.

tive predictive value of 73·8 (95% CI 60·9–84·2%), and a negative predictive value of 91·9% (95% CI 85·6–96·0%). (Fig. 4).

Among the 264 patients, 80 patients had a cryptogenic stroke. Three patients died within the first month of follow-up. These patients had NT-proBNP values of 1066, 1259, and 2426 pg/ml. In 17 (21·3%), pAF was found during follow-up. In 14 patients, the identification of pAF was done within the first month after stroke onset. There were no adverse events from the diagnostic tests. Patients in whom pAF was subsequently found were older and had higher admission NIHSS scores and NT-proBNP at admission than patients with no diagnosis of pAF (Table 3). In patients with cryptogenic stroke, the cutoff point of 265·5 pg/ml had a sensitivity of 88·2% (95% CI 65·7–96·7%), a specificity of 61·9% (95% CI 49·6–72·9%), a positive predictive value of 38·5% (95% CI 23·4–55·4%). and a negative predictive value of 95·1% (95% CI 83·4–99·3%) for the diagnosis of pAF. The cutoff point of 912 pg/ml had a sensitivity of 47·1% (95% CI 26·2–69·0%) and a specificity of 88·9% (95% CI 78·8–94·5%), a positive predictive value of 53·3% (95% CI 26·7–78·7%), and a negative predictive value of 86·2% (95% CI 75·3–93·5%) (Fig. 4). The AUC of the ROC curve of NT-proBNP for the diagnosis of pAF in patients with cryptogenic stroke was good (−0·83; 95% CI 0·73–0·92) (Fig. 3).

Discussion

NT-proBNP levels at the time of stroke onset were helpful to identify pAF on follow-up of patients with an undetermined stroke etiology. The previously defined cutoff points of 265·5 pg/ml and 912·0 pg/ml had a high sensitivity and specificity for the diagnosis of pAF in patients with cryptogenic stroke (88·2 and 88·9% respectively). This is the first study in which

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NT-proBNP accuracy in diagnosing pAF was analyzed in cryptogenic stroke and in which previously defined cutoff levels were evaluated and validated.

Normally, atrial and ventricle myocytes express BNP genes that code for proBNP. ProBNP is stored in atrial granules. Following the hemodynamic effect of AF, the precursor molecules (preproduced proBNP) that are stored in atrial myocytes are secreted as BNP and NT-proBNP (19), leading to high levels of NT-proBNP. Therefore, NT-proBNP levels could remain elevated in serum during sinus rhythm after a short course of pAF. Most likely, patients with cryptogenic stroke in whom AF was later found had an episode of AF close to stroke onset that caused an increase in NT-proBNP levels. Although they returned to a sinus rhythm, NT-proBNP remained elevated as a consequence of the episode of pAF. NT-proBNP has been shown to be a remarkable predictor of incident AF in the general population, independently of any other previously described risk factor (20). In our study, in a multivariate analysis, NT-proBNP was also related to AF independently of age, sex, atrial dilatation, systolic dysfunction, or left ventricular hypertrophy. There are recent publications that suggest that NT-proBNP is a predictor of AF following cardiac surgery (21–23) and successful cardioversion (24).

One report (24) evaluated NT-proBNP as a predictor of AF in patients with stroke in sinus rhythm in general and found that it had a reasonable accuracy for this diagnosis (AUC 0·638; 95% CI 0·531–0·744). Differences between this study and ours may explain the dissimilar result:

- In our study, we evaluated the accuracy of NT-proBNP in diagnosing AF in patients with cryptogenic stroke, while the study by Wachter *et al.* (25) evaluated NT-proBNP accuracy in diagnosing AF in stroke patients with sinus rhythm. The most clinically relevant question is which patients with cryptogenic stroke have AF. In the study of Wachter *et al.*, patients with an already established cardioembolic etiology were included in the group of patients further evaluated for AF (25). The cardioembolic etiologies of these patients were not included in the report (25). These already known cardioembolic etiologies may have contributed to increased NT-proBNP levels in these patients.
- We used different methods and longer time periods to look for AF, which might have influenced the results and led to a higher rate of detection of AF (12·7 vs. 21·3% in our study).
- In our study, we preferred to use NT-proBNP instead of BNP because NT-proBNP is known to have an increased half-life (19) and therefore may have a longer time window during which its levels can be determined after stroke onset. This is clinically important because a sizable fraction of patients do not go to the emergency department in the first 24 h after stroke onset.

Strengths of this study include the following:

- It is the first study that evaluated the accuracy of NT-proBNP in diagnosing AF in patients with cryptogenic stroke. For this, a follow-up time of six months was used.
- It was designed to take place in a clinical setting similar to the one in which it could be applied.
- This study corroborated the cutoff values for NT-proBNP that were defined previously as being very sensitive and specific for pAF.
- The STARD criteria were followed.

Limitations of this study include the time points during the follow-up after ischemic stroke that were chosen to look for AF. It is possible that in some patients with pAF, this arrhythmia was not diagnosed with the currently used diagnostic examinations. However, a low detection rate would only contribute to decreasing the sensitivity and specificity of the chosen points. Therefore, specificity might have been underestimated in this study. In patients with cryptogenic stroke, the diagnosis of AF is particularly important as it changes the therapeutic decision, leading to the prescription of anticoagulants instead of antiplatelet agents. Cryptogenic stroke patients are often subjected to extensive and costly electrophysiological investigations. NT-proBNP measurements could be helpful to guide or eliminate further ECG, Holter, or other electrophysiological monitoring in patients with stroke. In patients with cryptogenic strokes who have unremarkable ECGs, an increase in NT-proBNP may point to the presence of pAF and lead to further electrophysiological investigation.

Conclusion

NT-proBNP had good accuracy in predicting pAF in patients with cryptogenic stroke and might help in the evaluation of these patients.

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